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CHEMICAL AND MICROBIAL ASPECTS OF SLUDGE COMPOSTING AND LAND APPLICATION

by

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16. ABSTRACT

A series of six studies was conducted to evaluate selected microbial and chemical aspects of composting and sludge spreading. Ammonia (NH_3 was shown to be viricidal at pH 7 to 9). Enzymatic activity was not inhibited when well stabilized or composted sludge containing high concentrations of metals and chlorinated hydrocarbons was added to soils. Nitrogen and phosphorus moved down the soil profile where plots were treated with 100 Mg/ha once or 20 Mg/ha five times. Laboratory studies of composting showed that controlled aeration to maintain temperatures between 50 and 55°C allowed thermophilic decomposition to remain constant, produced more drying and CO_2 than constant aeration composting. *Aspergillus fumigatus* and thermophilic actinomycetes, low in non-agricultural sites, are common around composting and crop residues.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution, and it involves defining the problem, measuring its impact, and searching for solutions. The Water Engineering Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and is a most vital communications link between the researcher and the user community.

Potential soil contaminants such as wastewater sludge may become beneficial soil amendments when they are properly treated and applied to land. This research report discusses controls of human pathogens in sludge and sludge compost, analysis of the toxic effects of sludge by monitoring key biochemical reactions in soil, potential pollution of groundwater by nutrients from sewage sludge application, and development and use of a laboratory apparatus to evaluate key factors affecting the efficient composting of sewage sludge.

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Director
Water Engineering Research
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ABSTRACT

A series of six studies was undertaken to study various chemical and microbial aspects of the composting and land application of sewage sludge. Topics for study included viral inactivation by ammonia, effects of sludge and compost on soil enzymes, effects of sludge applications on nitrogen mineralization and phosphorus mobility in soils, effects of temperature and aeration on composting, and growth factors for Aspergillus fumigatus and natural sources of the airborne fungus and thermophilic actinomycetes.

This report covers several aspects related to the application of sludge to land and the composting of sewage sludge. Ammonia which is generated in sludge by ammonifying microorganisms was shown to be virucidal. In the pH range studied (7 to 9), NH_3 but not OH^- or NH_4^+ was effective. Temperature effects were related to a single NH_3 concentration. Arrhenius plots of the data were biphasic indicating that two inactivation processes were occurring -- one for the low-temperature range and another for the high-temperature range.

Studies of the effects of sludges on soil enzyme activities indicated that the stabilization level of the sludge correlated negatively with enzyme activity. Sludge and sludge compost with high concentrations of heavy metals and chlorinated organic materials appeared to have no inhibitory effect on soil enzyme activity, probably because of the age or the highly stabilized nature of the sludge and sludge compost.

In one study, sludges were applied once (100 Mg/ha) or annually (20 Mg/ha) for five years. N movement down the soil profile occurred in the sludge-amended plots, especially in all of the split plots that received additional fertilizer. Phosphorus movement occurred only down to the 60 cm depth or four times the depth of incorporation in the sludge-amended plots.

Laboratory studies were conducted to test methods for improving the efficiency of composting. Constant, high aeration did reduce peak temperatures, but it did not result in the most efficient composting system. Constant, high aeration removed water and cooled the mass rapidly. The most efficient system was the temperature demand aeration system. Increasing and decreasing the aeration rates to keep temperatures between 50 and 55°C allowed the maximum thermophilic population to remain active for extended periods of time, produced twice as much drying, and yielded 2.5 times as much CO_2 as constant aeration composting.

Results of studies have shown that Aspergillus fumigatus (AF) and thermophilic actinomycetes (TA) are present in air at very low levels in

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most nonagricultural sites even when organic matter is present and temperatures are occasionally higher than ambient. Thus the natural sources for AF and TA are very limited in terms of abundant production of spores. Compost and moldy agricultural substrates are the biggest reservoirs and sources of AF and TA. The tolerance of AF to broad ranges of temperature, CO_2, and water content limit the benefits of modifying the composting process by these parameters to inhibit AF.

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SECTION 1

INTRODUCTION

Applying sewage sludge or sludge compost to land can affect several chemical and microbiological factors in soils. The benefits of this practice, which include the addition of nutrients, trace metals, and organic matter, can be offset by excess additions of phytotoxic metals, nitrogen, and organic chemicals. The need to differentiate between proper and excessive additions to soil is imperative to evaluate properly the benefits of each sludge product. Certain sludges have excessive amounts of toxic materials and should not be applied to land. Other sludges may have elevated amounts, but they have no apparent phytotoxic consequences when added to soil at low rates . The long-term effects on soil productivity, surface water, and groundwater need to be evaluated as well as the short-term, first year effects.

This report will discuss the effect of NH_3 on the survival of viruses and the comparison of NH_3 to halogens for virucidal properties. The availability of sludge N and P to plants and the movement of these nutrients through the soil profile over the long-term will be discussed. Soil enzymes, the key to successful biochemical transformations in soil, were monitored for effects by large or repeated sludge applications. A better understanding of long-term effects will be put forward from the data presented here.

Composting stabilizes sewage sludges and transforms them into an easily spread and stored material. The composting process was analyzed extensively using a self-heating laboratory composter, and the effects of temperature and aeration on the efficiency of the composting process were determined. A better understanding of the optimization of the composting process for production of a material used as a soil amendment was obtained.

Composting, being a thermophilic process, results in a product containing numerous thermophilic organisms that have various public health implications. This report will provide data on numbers, types, and potential health implications of thermophilic organisms produced during composting of sewage sludge, during the movement of compost and other self-heating materials, and eventual distribution of these materials to users.

This project was conducted under an Interagency Agreement between the U.S. Environmental Protection Agency and U.S. Department of Agriculture and encompasses research that was initiated in 1977 and concluded in 1983.

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SECTION 2

CONCLUSIONS

Ammonia was shown to be virucidal in sludge and in NH_4Cl solutions. Temperature effects on NH_3 activity were biphasic indicating that two inactivation processes were occurring, one for the low temperature range and another for the high temperature range. The f2 virus was 5.4 times more affected by NH_3 than poliovirus 1. As a virucide, NH_3 is extremely weak compared to chlorine. Because of its high reactivity with organic material, disinfecting sludge with chlorine requires high dosages which translates into high chloride levels in the final sludge. The use of NH_3 to disinfect wastewater treatment plant effluents is precluded by the slowness of its reaction, but in sludge, NH_3 may indeed be a practical virucide.

The effects of 2 types of sludges and their corresponding sludge composts on soil enzyme activities indicated that the stabilization level of the sludge correlated negatively with enzyme activity. One sludge that was stored in a lagoon for several years and contained high concentrations of heavy metals and chlorinated organic materials showed no significant inhibitory effect on soil enzyme activity, probably because the sludge and the corresponding sludge compost were highly-stabilized with the toxic components either degraded or bound to organic matter.

Soil profile data indicated that when a digested sewage sludge was applied once at 100 Mg/ha rate or annually at 20 Mg/ha for five years, mineralized N in excess to plant needs was found below the root zone. The addition of fertilizer N to split plots increased the level of mineralized N in the deep soil profile. Phosphorus in the sewage sludge also migrated down the soil profile but not to the same depths as N. The 20 Mg/ha application rate was in excess to the nutrient needs of barley.

A self-heating laboratory composter was designed, built, and used successfully in studies conducted to test methods for improving the efficiency of composting. The most efficient composting system tested was one that adjusted aeration to hold temperatures near or below $55^{\circ}C$. Under this system, 2.5 times more decomposition and 2 times more moisture loss occurred from the compost than with a compost system using a constant rate of aeration. The loss of N from the compost was directly related to the efficiency of removing moisture from the compost.

Aspergillus _fumigatus_ (AF) and thermophilous actinomycetes (TA) are present in air only at very low levels in most nonagricultural sites even if organic matter and occasionally higher than ambient temperatures are present also. Thus, the natural sources for AF and TA are very limited in terms of abundant production of spores. Compost and moldy agricultural substrates are the biggest reservoirs and sources of AF and TA. The tolerance of AF to broad ranges of temperature, CO_2, and water content limit the benefits of modifying the composting process by these parameters to inhibit AF.

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SECTION 3

RECOMMENDATIONS

Ammonium was shown to be virucidal to f2 bacteriophage and poliovirus 1. The effect of NH_3 on the inactivation of several human pathogens needs to be evaluated before recommendations on the use of NH_3 as a means of reducing pathogen numbers in sewage sludge are made.

Caution should be exercised in evaluating the effects of heavy metals and chlorinated organic materials in sewage sludge on soil biochemical properties. Factors such as age of the sludge and the sludge treatment process should be considered in evaluating the effects of chemicals which, as salts, are known to be toxic to biological processes.

Application of sewage sludge to agricultural land should be coordinated with the crop's fertilizer need. Nitrogen mineralization rates of the sludge and soil should be determined and application rates adjusted accordingly. Application rates based on P mineralization and movement should be considered in soils such as sands which do not fix appreciable amounts of P.

A composting system should be designed to be as efficient as possible, i.e., result in a stabilized, dry product in as short a period of time as possible. Controlling temperatures so that they do not exceed 55 to 60 C by adjusting aeration provides a highly efficient composting system. Adequate destruction of pathogens under such a system, however, needs to be evaluated in laboratory and field trials.

Practical alteration of sewage-sludge composting process to reduce *Aspergillus fumigatus* growth and dispersal from compost sites presently should focus on use of noncellulosic bulking agents. Such agents would substantially reduce the inoculum levels entering the process.

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SECTION 4

KINETICS AND THERMODYNAMICS OF VIRAL INACTIVATION BY AMMONIA

INTRODUCTION

Virus Inactivation by NH_3

Ammonia has been shown to be toxic to a variety of organisms ranging from bacteria to mammals (Taylor et al., 1978; Warren, 1962). The virucidal capability of NH_3 was reported in digested sludge (Ward and Ashley, 1976) and later in undigested sludge (Ward and Ashley, 1977). Poliovirus 1 (Chat), Poliovirus 1 (Mahoney), Poliovirus 2 (712), Coxsackievirus A13, Coxsackievirus B1 and Echovirus II all have single stranded RNA and were all similarly affected by the activity of NH_3, but Reovirus 3 has double stranded RNA and was found to be more resistant (Ward and Ashley, 1977). In studying the mechanism of poliovirus inactivation, Ward (1978) found that inactivation by NH_3 caused a slight reduction in the sedimentation coefficients of viral particles, but had no effect on the electrophoretic pattern of viral capsid proteins or the isoelectric points of intact particles. RNA was cleaved but only when present as a component of intact virus particles. Naked RNA appeared to be unaffected. In the work of Ward and associates the kinetics of the inactivation of viruses as influenced by NH_3 concentration was not studied nor did the nature of the experiments allow separation of the effects of NH_4^+ from OH^-.

Fenters et al. (1979) confirmed the virucidal activity of NH_3 in wastewater sludge and studied the effect of NH_3 concentration on the Sabin strain of poliovirus 1 at 35°C. A positive correlation was found between NH_3 concentration and rate of virus inactivation. However, as in the studies of Ward and his associate, the experimental procedures were not such that the effect of NH_4^+ and OH^- could be distinguished from that of NH_3.

Heating has been shown to increase the rate of viral inactivation by NH_3 in sludge (Fenters et al., 1979; Ward and Ashley, 1977), but the relationship between heat and NH_3 inactivation has not been described mathematically. When applied to the thermoinactivation of viruses, the magnitude of the energy of activation (E), the changes in the enthalpy of activation ($\Delta H^\ddagger$), and the entropy of activation ($\Delta S^\ddagger$), can be used to help deduce the nature of the reaction (Ginoza, 1958; Woese, 1960).For single-stranded RNA viruses, large values of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ indicate that the viral coat is the site of inactivation through protein denaturation while smaller values may mean that RNA is the site through rupture of the RNA chain. For inactivation by NH_3, small values

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would tend to reinforce Ward's (1978) observation that RNA cleavage was involved in poliovirus inactivation while large values would tend to indicate that RNA cleavage was incidental.

In this study, the kinetics of f2 and poliovirus 1 (Chat) inactivation by NH_3 were compared under conditions in which the effect of NH_3, NH_4^+ and OH^- concentrations could be separated at 20°C. Then for thermodynamic calculations the kinetics of inactivation were followed for f2 at 40, 50, and 60°C and for the poliovirus at 40 and 50°C at one NH_3 concentration (300 mg/L). The data obtained for the effect of temperature along with those at 20°C were used to determine E, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$.

MATERIALS AND METHODS

Growth Purification and Assay of f2

The bacterial virus was obtained from the American Type Culture Collection (ATCC #15766-B) as was its host *Escherichia coli* K-13 (ATCC #5766). The host was grown as described by Loeb and Zinder (1961) in tryptone yeast extract medium. Ten liter batches of f2 were prepared in a fermenter at 37°C under aerobic conditions. The medium was inoculated with an overnight culture of K-13. At an absorbance of 0.4 (5×10^8 cells/mL), the appropriate amount of f2 was added to give a multiplicity of infection (MOI) of 0.1. After bacterial lysis had stopped, 20 mL of chloroform, 1 g of lysozyme and 300 mL of 0.2 M ethylenediaminetetraacetic acid at pH 7.5 were added and mixed for 10 min. The bacterial debris was removed by centrifugation. The f2 preparations, which averaged 2.0×10^{12} plaque forming units (pfu) and were stored at 4°C.

The virus in the clarified lysate was concentrated by the polyethylene glycol method of Yamamoto et al. (1970). The precipitated virus was resuspended in 0.01 M phosphate buffered saline (PBS) adjusted to pH 7.4, and then centrifuged at 15,000 x g for 10 min. The virus in the supernatant fluid was pelleted by a 2-h spin at 100,000 x the force of gravity (g) using a No. 40 rotor. After the addition of CsCl (0.64 g/cm^3) the gradient was centrifuged at 100,000 x g in a No. 40 rotor for 22 h. The CsCl gradient was fractionated, and the 2-3 fractions contained in the peak were pooled and then dialyzed against PBS for 24 h with 3 changes in fluid. Samples were assayed for f2 by the agar overlay method described by Adams (1959) in 100 mm Petri dishes.

Growth Purification, and Assay of Poliovirus

The attenuated Chat strain of poliovirus 1 (ATCC #VR-192) was grown in the serially propagated Buffalo Green Monkey Kidney (BGMK) cells obtained from Microbiological Associates, Walkersville, MD, in the twenty-fourth passage. The procedures for growing the virus were identical to that of previously reported (Cramer et al. 1976) except that Eagle's minimum essential medium with Earle's salts was used instead of Hank's. The titer of the fifth passage was 2.2×10^8 pfu/ml.

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Poliovirus preparations were purified using a slight modification of the procedures reported by Henry and Youngner (1963) and Floyd et al. (1976). Five to ten 150 cm^2 flasks of BGMK cells were infected with the virus at an MOI of 20. After 11 h at 37°C the cells were chilled to 4°C and the medium decanted. The monolayers were washed twice with Dulbecco's phosphate buffered saline (DPBS) and then scraped into Mg^{++} and Ca^{++} free DPBS. An equal volume of Freon 113 was added, and the mixture was homogenized for 2 min at room temperature in a Sorvall Omnimixer at half speed. The mixture was centrifuged at 800 x g for 10 min and the water phase containing the virus removed. The Freon phase was re-extracted and the two water phases were pooled. With the use of a No. 30 rotor, the virus was pelleted by a 4 h spin at 66,000 x g. The pellet was suspended in PBS, clarified as before and after the addition of CsCl (1.34 g/cm^3) was spun at 80,000 x g for 22 h in a No. 40 rotor. The gradient was fractionated and the 2-3 fractions were pooled and dialyzed against PBS for 24 h with three changes in fluid.

Samples were assayed for poliovirus by the plague method in 25 cm^2 polyethylene tissue culture flasks as described previously (Cramer et al., 1976).

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Preparation and Assay of NH_3 Solutions

NH_4Cl was dissolved in sterile 0.1 M tris-(hydroxymethyl) aminomethane (TRIS) made with ammonia free water and adjusted to pH 6.0 with 1 M HCl. NH_3 working solutions were prepared by adding appropriate amounts of the stock solution to sterile 0.1 M TRIS which was then adjusted to the experimental pH with 0.5 M NaOH. The working solution was prepared immediately before the start of an experiment and checked for NH_3 concentration before use. The concentration of NH_3 at the experimental pH was determined in the stock and other solutions with a specific-ion electrode. Upon determination of the level of NH_3 in the sample, the total ammonia nitrogen was found by adding enough 10 N NaOH to raise the pH to 12.5. The concentration of NH_4^+ was determined by subtracting the NH_3 value from the total ammonia nitrogen.

Experimental Procedure

In all studies 199 mL of the ammonia working solution were poured into a sterile 250 mL Erlenmeyer screw capped flask containing a small magnetic stirring bar. The purified f2 or poliovirus stock solutions were diluted appropriately in distilled water and 1 mL of the solution was added to give a titer in the flask of from 2.1×10^6 to 2.0×10^7 pfu/mL. The contents of the flask were mixed for 1 min after which a portion was removed to check the initial virus titer, the pH and the ammonia level. The flask was tightly capped and incubated at the desired temperature in a low temperature incubator previously adjusted to the temperature being used. The flasks were placed into the incubator for a sufficient amount of time for the contents to equilibrate with the experimental temperature before the virus was added. Solutions were sampled at appropriate times to check for changes in pH or ammonia level and to evaluate virus survival. All experiments were repeated again on a different day.

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Control

The control solutions were prepared in the same way as described above so that the same pH would be achieved except that the NH_4Cl was replaced by an equivalent amount of NaCl so that the ionic strength of both would be identical. The data from the treatments were corrected for losses in the control by the following equation,

$$\text{Percentage of virus surviving} = \frac{N}{M} \times 100 \qquad (1)$$

in which N is the number of virus particles surviving exposure to ammonia, and M is the number surviving in the control flask at the same time interval.

Inactivation of f2 and Poliovirus

NH_3 Concentration Controlled by pH (f2) --

The f2 phage was inoculated into flasks of 0.25 M NH_4Cl and NaCl adjusted to pH 7.6, 8.1 and 8.6. At these pH's the NH_3 concentrations in the NH_4Cl flasks were 22, 70, 215 and 617 mg/L, respectively.

NH_3 Concentration Varied With pH Held Constant (f2 and Poliovirus) --

Studies were carried out with f2 at pHs of 7.0, 8.0, and 9.0. The NH_3 concentrations were 10, 30 and 80 mg/L at pH 7.0, 50, 100, 300 and 800 mg/L at pH 8.0 and 500, 1000 and 3000 mg/L at pH 9.0. The NH_3 levels were adjusted by adding enough NH_4Cl to produce the desired NH_3 concentrations at the various pH levels. NaCl equivalent to the NH_4Cl additions was added to the controls. The amount of NH_4Cl needed for addition was estimated by the Henderson Hasselbalch equation, and the actual amounts produced were measured by use of a specific-ion electrode. The studies with poliovirus were carried out in the same way, but only pH 8.0 was used.

pH Varied with NH_3 Concentration Held Constant (f2 and Poliovirus) --

f2 bacteriophage and poliovirus were exposed to a concentration of 100 mg/L NH_3 at pHs 6.5, 7.0, 8.0, and 9.5.

Temperature varied (f2 and Poliovirus) --

In these studies the NaCl and NH_4Cl solutions were adjusted to pH 8.0. The bacterial virus was exposed to NH_3 levels of 25, 50, 150, and 400 mg/L at 10°C and 40, 100, 200, and 600 mg/L at 30°C. Poliovirus was dosed with 50, 100, 300, and 800 mg/L of NH_3 at both temperatures. For the higher temperature studies of 40, 50 and 60°C for f2 and 40 and 50°C for poliovirus, an NH_3 concentration of 300 mg/L was used. A temperature of 60°C for poliovirus was attempted, but the inactivation rate was too rapid to obtain survival data.

For thermodynamic calculations the inactivation rate constants (k) for 300 mg/L of NH_3 were used. Since this concentration was not used in the kinetic studies for f2 at 10 and 30°C, the k for 300 mg/L was calculated from the relationship experimentally obtained for k and NH_3 concentrations actually used.

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RESULTS

NH_3 Concentration Controlled by pH

The rate of inactivation of f2 was greatly accelerated by increasing the NH_3 concentration through elevation of the pH (Fig. 1). The pH effect is cancelled by calculating the survival relative to survival in a control of the same ionic strength and at the same pH. In these studies and the others reported herein, the pH once established did not change more than ± 0.05 pH units throughout the experimental period. The kinetics of inactivation were clearly first order. The k values were −0.0039, −0.0111, −0.0162, and −0.0699 h^{-1} for the pH values of 7.1, 7.6, 8.1, and 8.6, respectively. A test for significance of linearity of regression showed that the curve at pH 7.1 was linear at the 5.0% level. The rest were significantly linear at the level of 1% or lower.

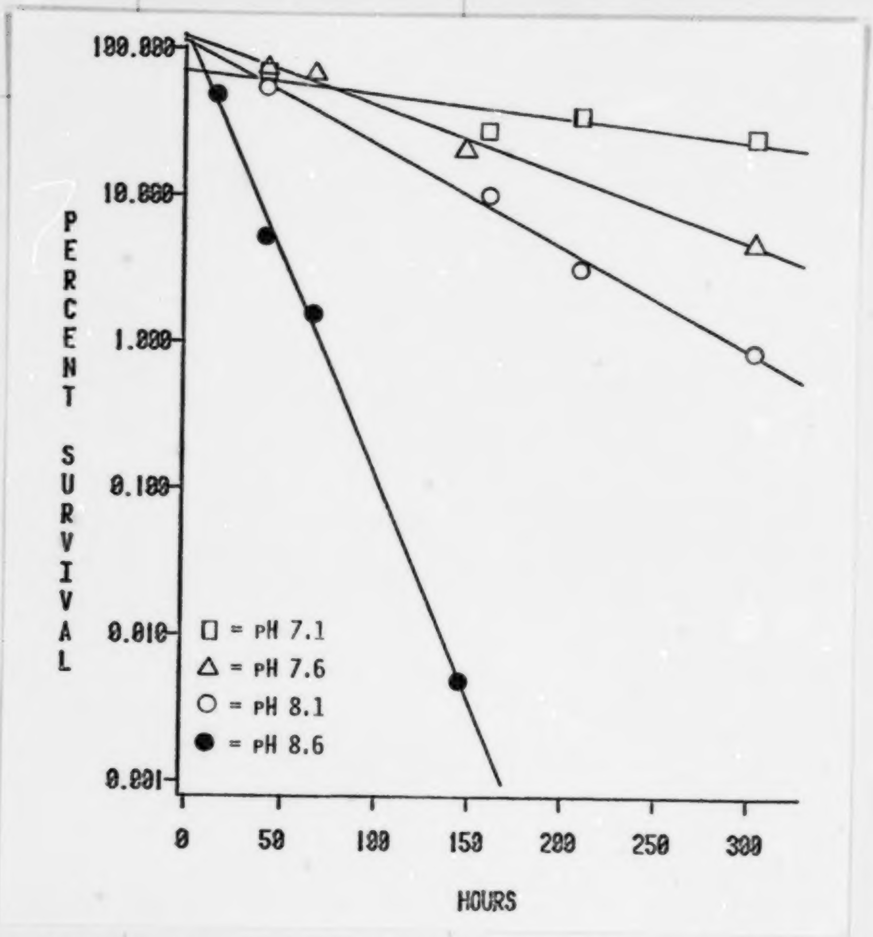

Fig 1. Inactivation of f2 bacteriophage in 0.25 M NH_4Cl solutions at 20°C adjusted to pH values of 7.1, 7.6, 8.1, and 8.6 containing 22, 70, 215, and 617 mg/L of NH_3, respectively.

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NH_3 Concentration Varied and pH Held Constant (f2 and Poliovirus)

The survival curves for both f2 and poliovirus at pH 8.0 were highly linear (Fig. 2). Survival curves for f2 (not shown) at pH 7.0 and 9.0 were also highly linear. For both viruses, survival is greatly decreased at constant pH as free NH_3 concentration is increased.

The log k value for the inactivation of poliovirus at pH 8.0 and the log k values for inactivation of f2 at pH 7.0, 8.0, and 9.0 were found to be linearly related to the log NH_3 concentration (Fig. 3). The log k values for f2 fell on a single line despite the variation in pH showing that the effect of OH^-, if any, was very small relative to the effect of NH_3.

The greater sensitivity of poliovirus 1 to NH_3 is evident in Fig. 3. The plots of the data are linear for both viruses and can be considered parallel ($P > F = 0.01$). The antilog of the difference between the coordinates in midrange can be taken as a ratio of the sensitivity of the two viruses to inactivation by NH_3. The value of 4.5 is a measure of how many times more sensitive poliovirus 1 was than f2 to inactivation by NH_3.

pH Varied and NH_3 Concentration Held Constant (f2 and Poliovirus)

The additional check on the effect of NH_3 versus OH^- on the inactivation of f2 and poliovirus 1 (Chat) made by varying pH values and holding the NH_3 concentration constant, also indicated no significant effect for OH^-. The respective k values at pH's 6.5, 7.0, 8.0, and 9.5 for f2 were -0.022, -0.035, -0.032, and -0.0278 h^{-1}. At the same pH

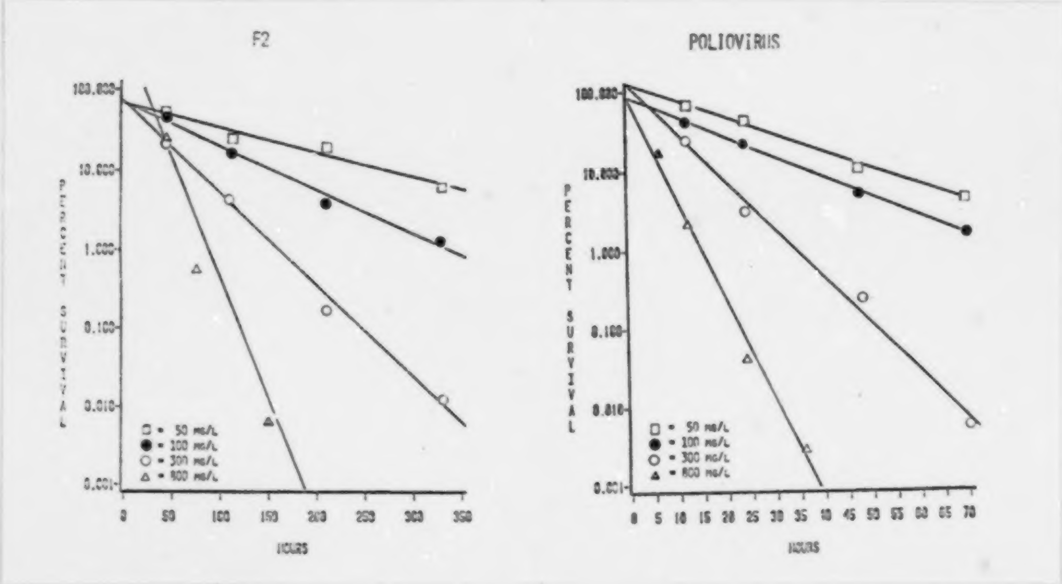

Fig. 2. Inactivation of f2 bacteriophage and the Chat strain of poliovirus 1 by NH_3 at 20°C.

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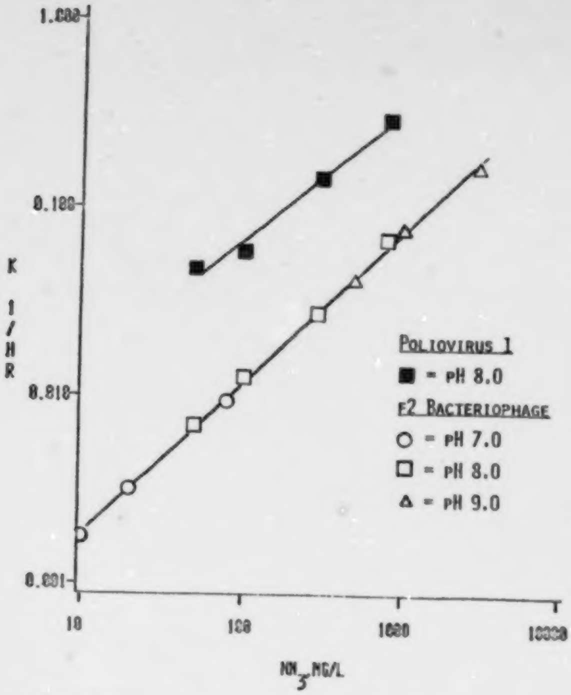

Fig. 3. Effect of OH^- concentration on the inactivation of f2 bacteriophage at 20°C.

values, the k values for poliovirus were respectively, -0.097, -0.087, -0.104, and -0.108 h^{-1}. An analysis of the data showed all the regressions to be significantly linear at the 5% level or better. The 4 k values for f2 as well as those of poliovirus were not significantly different at the 5% level despite large variations in OH^- concentrations among the treatments. Again the experimental results indicate that NH_3 is instrumental in the inactivation process and that the effects of OH^- in the range of OH^- investigation are minor.

Effect of Control

An example of the effect of adjusting the virus survival data for increases in NH_4^+ concentration by use of controls containing Na^+ is shown in Fig. 4. For these log-log plots, inactivation rate constants taken from poliovirus and f2 survival curves (Fig. 2) adjusted by use of controls were plotted against their corresponding NH_3 concentrations. For comparison the rate constants from survival curves not adjusted by use of controls were plotted against their corresponding NH_4Cl treatment (NH_4^+ plus NH_3) concentrations. The NH_3 values were transformed by multiplication with the factor 2.51, which was arbitrarily chosen to place the values on the same scale so that the curves could be conveniently compared. The two lines for poliovirus were identical ($P > F = 0.05$), whereas those for f2 were not. Since the regressions were different for f2, an additional test was made to determine if adjustment by use of

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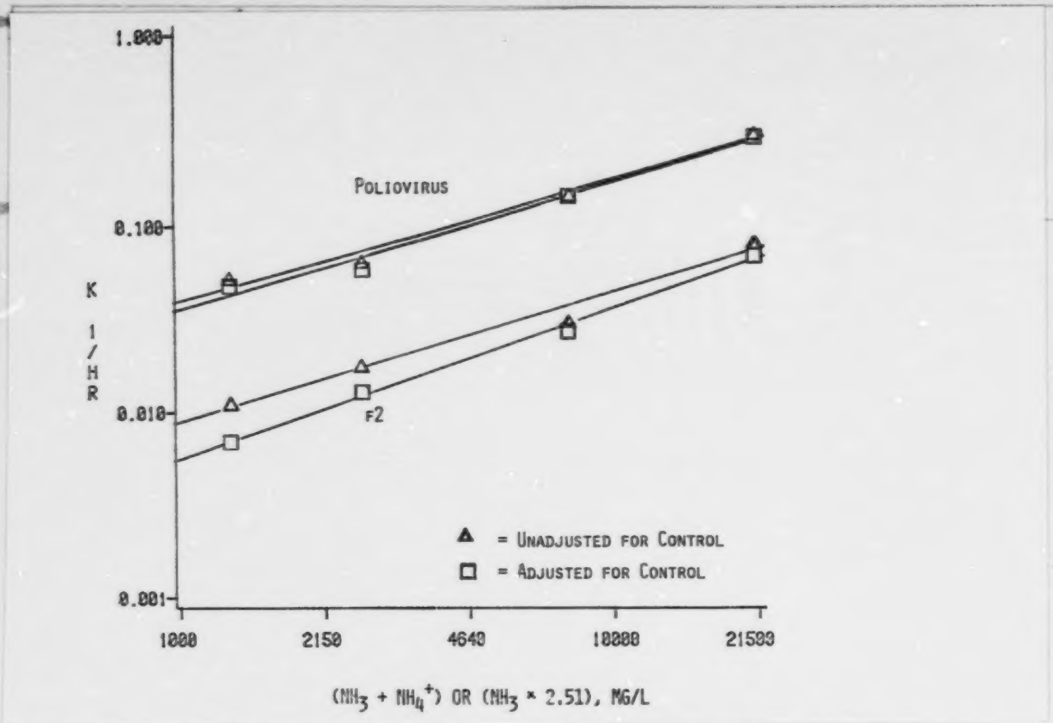

Fig. 4. Effect of NH_4^+ and NH_3 on the inactivation of f2 bacteriophage and poliovirus 1 (Chat) at 20°C.

controls improved the fit. The results of the two-tailed F test showed that there was not a significant reduction in the variance ($P > F = 0.05$) due to adjustment with the control.

The results of these tests show that the poliovirus inactivation rate was influenced much less, if at all, by the effect of NH_4^+ concentration than was the inactivation rate of f2. The convergence of the adjusted and unadjusted curves for both sets of virus data in Fig. 3 indicates that the relative effect of NH_3 increased as the NH_4^+ and NH_3 concentrations increased. This occurred even though the NH_3/NH_4^+ ratio essentially remained the same, since the pH was held constant.

Varying Temperature (f2 and Poliovirus)

The dependence of the rate of inactivation on NH_3 concentration of f2 (Fig. 5) and poliovirus (Fig. 6) is evident at the temperatures of 10 and 30°C just as at 20°C as previously shown. The curves are highly linear indicating again the pseudo-first-order nature of the reaction. The inactivation rates for f2 at 40, 50, and 60°C and for poliovirus at 40 and 50°C were also clearly pseudo-first order as shown by the linearity of the curves resulting from semilog plots (Fig. 7 and 8, respectively).

For both f2 and poliovirus, a plot including the 20°C data (2) of ln k as a function of ln (NH_3) produced a family of approximately parallel

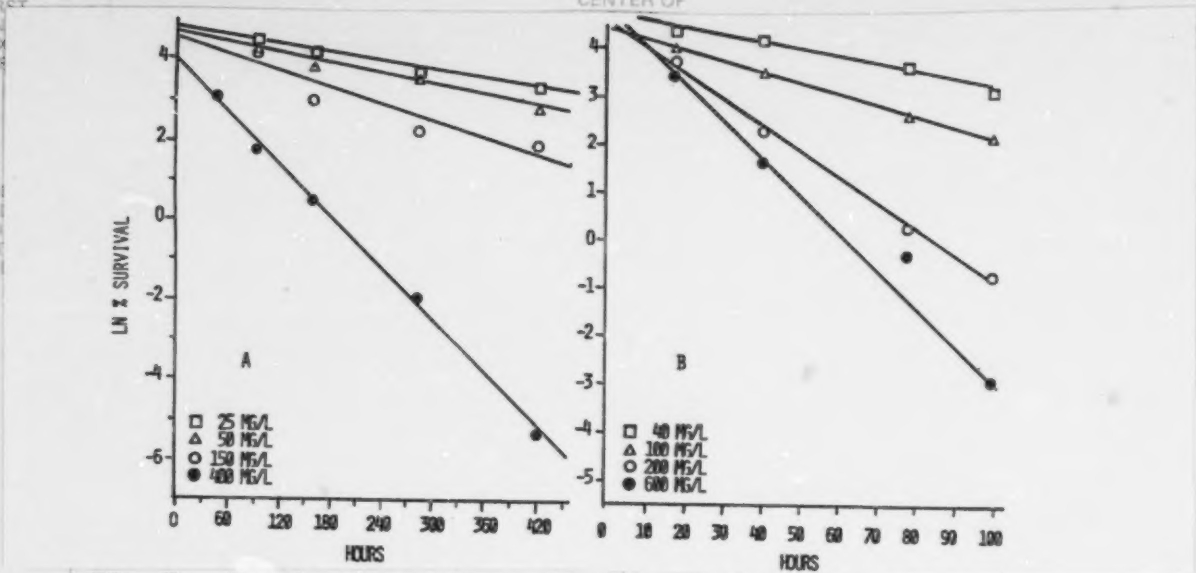

Fig. 5. Inactivation of f2 at 10°C (A) and 30°C (B) in NH_4Cl solutions containing NH_3 concentrations as shown.

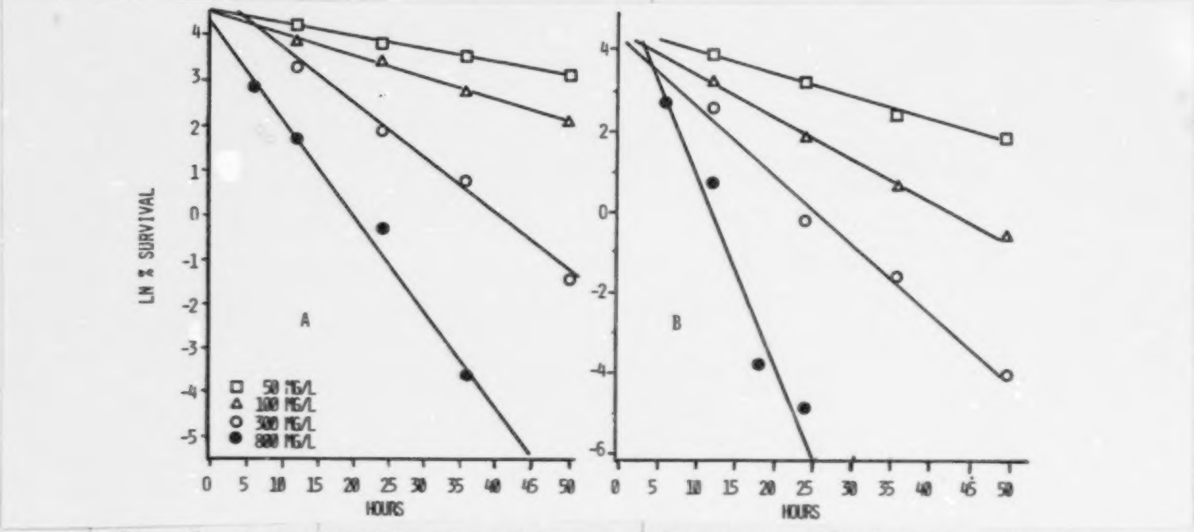

Fig. 6. Inactivation of poliovirus 1 (Chat) at 10°C (A) and 30°C (B) in NH_4Cl solutions containing NH_3 concentrations as shown.

lines (Fig. 9). F tests for the linearity of the log regressions showed that they were all highly significant. No interaction between NH_3 concentration and temperature was found when the slopes of the six lines were compared indicating they could not be considered other than parallel ($P>F = 0.05$).

It is apparent from Fig. 9 as from the survival curves that f2 is more resistant to NH_3 than is poliovirus. Even at 30°C the k values for f2 were less than those of poliovirus at all three temperatures studied. This

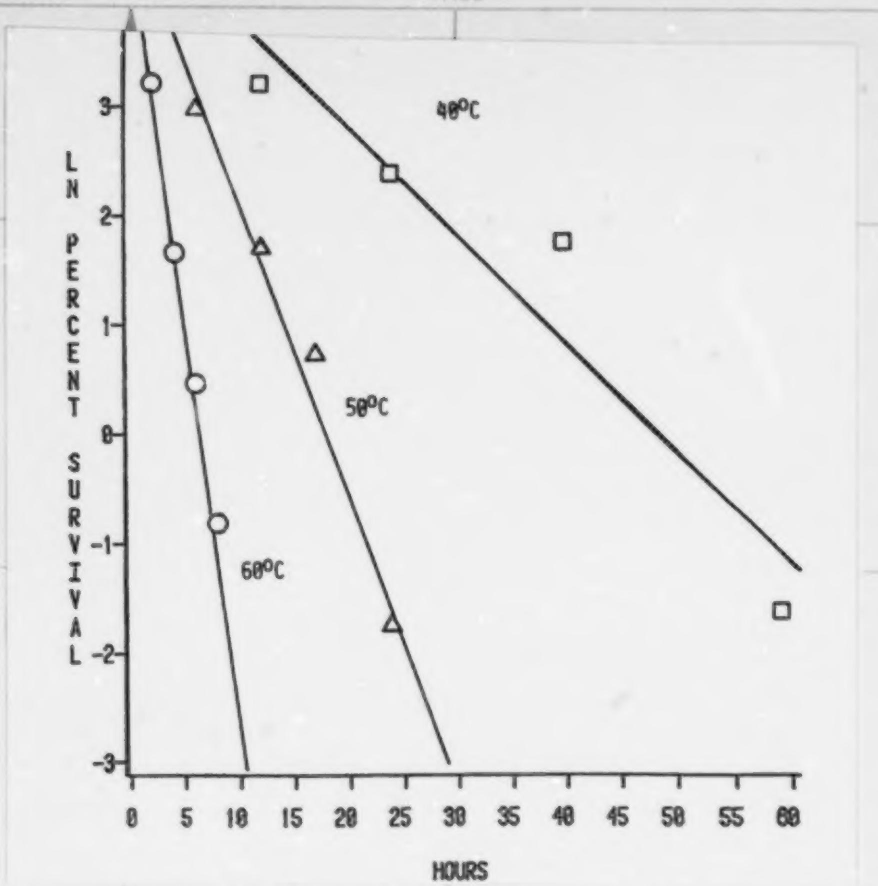

Fig. 7. Inactivation of f2 at 40, 50, and 60°C at NH_3 concentration of 300 mg/L.

can be shown another way by comparing the times needed by the two viruses for 99% inactivation at common NH_3 concentrations and temperatures. For this purpose the k values are divided into 4.606 (Fair and Geyer, 1954). As an example of the difference in the sensitivity of the viruses at 20°C and 100 mg/L NH_3, 363 hours were needed for inactivation of f2 compared to only 71 h for poliovirus 1.

Although f2 was more resistant to inactivation than poliovirus 1, the change in the rate of inactivation was greater with increasing temperature for f2 than for poliovirus in the lower temperature range. This is evident from the wider spacing in the lines relating to f2 than for poliovirus in Fig. 9 but can be more clearly shown by calculation of the Q_{10} values by the following equation

$$Q_{10} = \frac{k_2}{k_1} \exp \frac{10}{T_2 - T_1} \qquad (2)$$

in which k_1 and k_2 are inactivation rate constants for T_1 and T_2, respectively, as expressed in degrees absolute. The resulting Q_{10} values

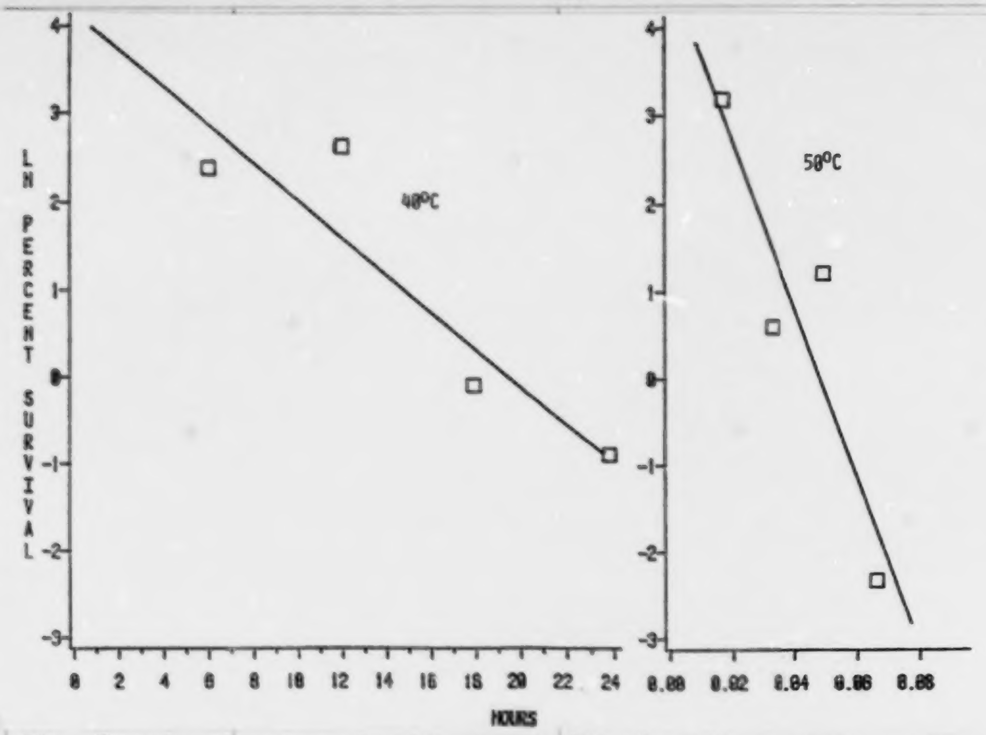

Fig. 8. Inactivation of poliovirus 1 (Chat) at 40 and 50°C at NH_3 concentration of 300 mg/L.

for f2 at 300 mg/L of NH_3 ranged from 1.57 between 10 and 20°C to 2.08 between 20 and 30°C with an average of 1.82. The values for poliovirus 1 under the same conditions respectively were 1.13 and 1.20 with a mean of 1.16.

The relationship between the rate of reaction constant and temperature can be seen in the Arrhenius equation shown in its integrated form,

$$\ln k = -E/RT + C \qquad (3)$$

in which T is the absolute temperature, E is the energy of activation and R is the molar gas constant (1.99 cal/°C). When ln k is plotted against 1/T, a straight line is generated with a slope of $-E/R$ and an intercept C on the ln k axis.

Further, in accord with Eyring's theory of absolute reaction rates (Daniels and Alberty, 1963), the change in the enthalpy of activation ($\Delta H^{\ddagger}$) is a function of E as follows:

$$\Delta H^{\ddagger} = E - RT, \qquad (4)$$

and $\Delta S^{\ddagger}$ is a function of $\Delta H^{\ddagger}$

$$\Delta S^{\ddagger} = R \ln \frac{k N_o h}{Z R T} + \frac{\Delta H^{\ddagger}}{T} \qquad (5)$$

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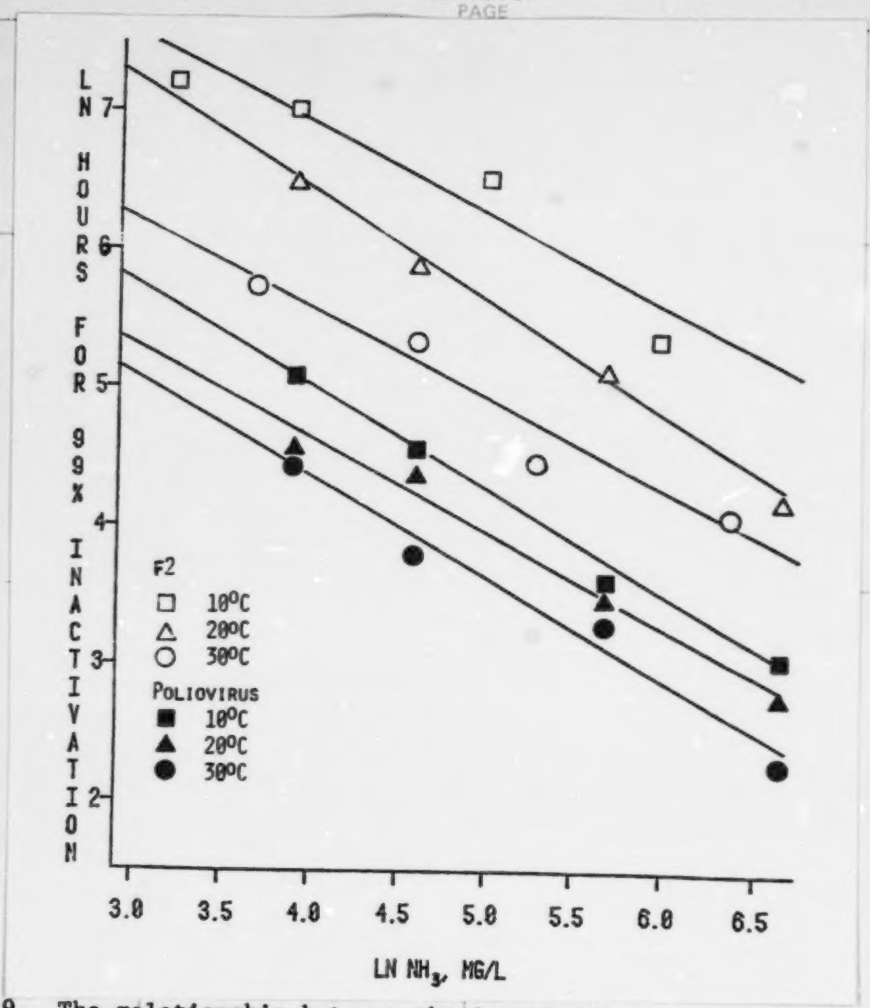

Fig. 9. The relationship between the inactivation rate constant (k) and NH$_3$ concentration as influenced by temperature.

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\,\Delta S^{\ddagger} \qquad (6)$$

wherein N_o is Avogadro's number, h is Planck's constant (6.625×10^{-27} erg s), and Z is the transmission coefficient usually taken to be one.

Arrhenius plots for f2 and poliovirus (Fig. 10) as made for NH$_3$ concentration of 300 mg/L show that inactivation by NH$_3$ is biphasic. In the lower temperature range the E is higher for f2 as indicated by a greater slope than for poliovirus. At the higher temperature phase, however, the situation changes dramatically with a much greater slope (higher E) for poliovirus. The values for E and the thermodynamic state variables ($\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$) as calculated by use of equations (3), (4), and (5), (6), from the slopes of the curves of Fig. 6 are given in Table 1.

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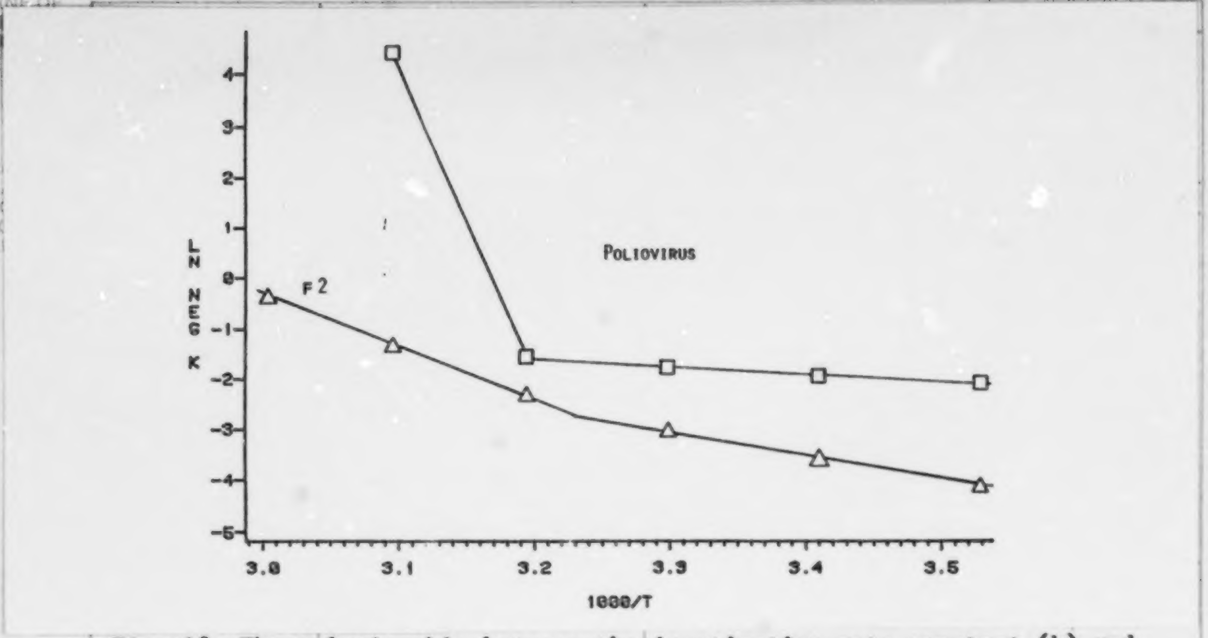

Fig. 10. The relationship between the inactivation rate constant (k) and temperature (° Kelvin) for f2 and poliovirus 1 (Chat).

TABLE 1. RATE CONSTANTS FOR TEMPERATURES USED AND CORRESPONDING THERMODYNAMIC STATE VARIABLES CHARACTERIZING THE BIPHASIC INACTIVATION OF f2 BACTERIOPHAGE AND POLIOVIRUS 1 (CHAT) BY NH_3 AT A CONCENTRATION OF 300 mg/l.

Virus	Temp. °C	k h^{-1}	E kcal/mole	$\Delta H^{\ddagger}$ kcal/mole	$\Delta S^{\ddagger}$ eu (cal/deg mole)	$\Delta G^{\ddagger}$ kcal/mole
f2	10	0.0160	9.30	3.66	-70.11	23.51
	20	0.0274	"	3.46	-70.25	24.05
	30	0.0488	"	3.27	-70.22	24.56
	40	0.0995	20.84	14.60	-33.06	24.95
	50	0.2699	"	14.40	-33.21	25.31
	60	0.7335	"	14.20	-33.18	25.25
Poliovirus	10	0.1245	3.19	2.63	-69.79	22.39
	20	0.1434	"	2.61	-69.92	23.11
	30	0.1719	"	2.59	-69.93	23.79
	40	0.2145	61.86	61.24	117.5	24.44
	50	96.5623	"	61.22	132.9	18.27

DISCUSSION

Kinetics of Inactivation

The semilogarithmic plots of the survival data (Fig. 2) were all linear for both viruses, indicating that the inactivation reaction was first order. The reactions must have been at least bimolecular, since it involved at least an NH_3 molecule and a vital site on the virion. Despite the involvement of both NH_3 and a site on the virus, only the decrease in virus concentration affected the kinetics, since the amount of NH_3 reduced was such an extremely small fraction of the amount present. Therefore, the reaction must be considered pseudo-first order rather than first order. With poliovirus and most likely, f2, the site is located in the nucleic acid (Ward, 1978).

Since OH^- had no effect, the rate equation can be written with only terms for the virus and NH_3 concentrations, $[V]$ and $[NH_3]$, respectively. For any $[NH_3]$,

$$v = k[V] \tag{7}$$

where v is the velocity of the reaction. As shown in Fig. 3, k is a log-log function of $[NH_3]$, and therefore,

$$k = k_o[NH_3]^b \tag{8}$$

where k_o is the antilog of the intercept, i.e., the value of k when $[NH_3] = 1.00$ mg/L and b is the slope of the line in Fig. 3. By substitution of the value of k in equation 8 for k in equation 7, the rate equation becomes:

$$v = k_o[V]\ [NH_3]^b \tag{9}$$

The values for k_o and b at 20°C were, respectively, 0.81 and 0.003 for f2 and 0.68 and 0.00029 for poliovirus.

The mechanism for the reaction of both viruses with NH_3 is proposed to be:

$$NH_4^+ \overset{K_1}{\rightleftharpoons} NH_3 + H^+ \text{, fast} \tag{i}$$

$$NH_3 + V \overset{K_2}{\rightleftharpoons} X\text{, slow} \tag{ii}$$

$$X \overset{k_3}{-\!\!-\!\!-} X^1\text{; slow and rate determining} \tag{iii}$$

where X is the activated complex, X^1 represents intermediates in the reaction, and K_1 and K_2 are equilibrium constants for the dissociation of NH_4^+ and X, respectively.

The total ammonia concentration, $[AM]_t$, is given in equation 10. Using the rate-determining equation and K_1 and K_2, an expression for the rate of virus inactivation at various $[AM]_t$ values can be derived:

$$[AM]_t = [NH_3] + [NH_4{}^+] + [X] \tag{10}$$

$$v = \frac{K_1K_2k_3\ [V]\ [AM]_t}{K_1 + [H^+] + K_1K_2\ [V]} \tag{11}$$

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Since K_2 is very much smaller than K_1, the equation can be simplified:

$$v = \frac{K_1K_2k_3\,[V]\,[AM]_t}{K_1 + [H^+]} \qquad (7)$$

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NH_3 is a virucide, but it is an extremely weak one compared to chlorine, the most commonly used disinfectant in the wastewater treatment process. Because of its high reactivity with organic material, disinfecting sludge with chlorine requires a very large dose. Heavily chlorinated sludge is often toxic to plants and may contain mutagenic substances (Kamlet, 1979). The use of NH_3 to disinfect wastewater treatment plant effluents is precluded by the slowness of its reaction, but in sludge, NH_3 may indeed be a practical virucide.

Although this study was conducted in solutions without sludge, we believe the results are relevant. The effect of sludge solids could be to protect viruses or possibly make some more susceptible to NH_3 or possibly through adsorption of NH_3, change the point of equilibrium. These effects, however, would most likely be minor.

The production of NH_3 by heterotrophic organisms from sewage sludges and other nitrogenous substances having relatively low C/N ratios is a natural process. The quantities produced usually result in pH elevation above neutrality and therefore significant quantities remaining in the NH_3 form. Nitrification proceeds slowly if at all so that the pH and NH_3 concentrations may remain relatively high for a considerable period. This would be true particularly if lime were added to help keep the pH elevated and the sludge was enclosed to inhibit volatilization of NH_3. The effect of NH_3 on the inactivation of other pathogens present in sludges needs to be determined.

Seeding with f2 may well be a practical approach to studies of virus inactivation in wastewater treatment plants. The data from this study and others indicated that f2, like the animal viruses tested so far, is inactivated by NH_3 but at a rate some 5.4 times slower than poliovirus 1 (Chat). Because f2 does not replicate in sewage sludge and serological methods are available for distinguishing it from endemic bacteriophages, it could make a very useful organism for estimating the rate of enterovirus inactivation under sludge storage and various field application or disposal systems.

Mechanism of Inactivation Derived from Thermodynamic Considerations

Values from the determination of the thermodynamic state variables can be used to help deduce the nature of a reaction. In the case of virus inactivation by heat, Woese (1960) states that for many of the simple virus particles the process reduces to either damage to receptor sites through denaturation of the protein coat or damage to nucleic acid or perhaps damage to both the coat and nucleic acid may be involved. The inactivation of protein and DNA is associated with high $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values because

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it is associated with the rupture of a number of hydrogen bonds whose breakage results in a collapse or drastic refolding of the molecules' secondary structure. Such a complex process might be expected to be higher than first order. However, heat inactivation studies (Greenberg et al., 1940) have shown that it is first order for the heat denaturation of a number of enzymes although not for all that were tested.

The energy involved is lower for single stranded RNA because the rupture of one diphosphoester bond linking nucleotides will cleave the molecule. For DNA and double-stranded RNA, the rupture of at least two bonds appears needed within a span of not more than two nucleotides (Ward and Ashley, 1970). The $\Delta H\ddagger$ accompanying inactivation of single stranded RNA may be on the order of ten-fold lower than for DNA and protein and the $\Delta S\ddagger$ values may not only be much lower but may be negative.

Negative $\Delta S\ddagger$ values indicate a loss in degrees of freedom, i.e., formation of a less probable structure due to molecular rearrangements or new bonds formed during the rupture of the old ones. Brown and Todd (1955) discussed a possible explanation of negative $\Delta S\ddagger$ values accompanying the inactivation of single-stranded RNA. Hydrolytic cleavage of the diphosphoester nucleotide linkage is preceded first by formation of a triphosphoester involving a reaction with the C_2'-OH, a group not present in DNA molecules.

The above reasoning is not totally applicable to inactivation by NH_3, because an inactivation reaction not involving RNA breakage (such a reaction might be one to block a receptor site in the virus protein coat) might possibly proceed with relatively low values for the thermodynamic variables. However, the low values for $\Delta H\ddagger$ and $\Delta S\ddagger$ make it not possible to rule out rupture of RNA as a possible mechanism at all the temperatures for f2 and for poliovirus below 40°C. Therefore, these results lend support, or, at least, do not contradict Ward's (1978) proposal that RNA rupture is the mechanism for inactivation. For poliovirus at temperatures above 40°C, the large size of the $\Delta H\ddagger$ and $\Delta S\ddagger$ values make it seem likely that inactivation occurs by some other process.

The values obtained for poliovirus for the high temperature range might be criticized on the basis that the slope utilized for calculation of E was obtained from a line drawn through only two points with the lower point appearing to fit well into the low temperature phase data (Fig. 6). However, if another point were obtained between the 50 and 40°C points, it could only have increased the slope. (An attempt was made to obtain survival data at 60°C for poliovirus but the inactivation rate was too rapid to obtain satisfactory measurements.) An increase in the slope would increase the values for the change in enthalpy and entropy of activation further supporting the conclusion that RNA rupture was not involved in the inactivation process.

The hypothesis that the relatively low energies observed in the inactivation of f2 throughout the entire temperature range studied and poliovirus in the lower range studied are associated with the vulnerability of single-stranded RNA is consistent with the observation by Ward and Ashley (1977). They observed in an NH_3 inactivation study using four single-stranded RNA viruses and one double-stranded RNA virus that only the double-stranded RNA one, a reovirus, was resistant to inactivation. This implies that DNA viruses because of their double-stranded nucleic acid may also be resistant to inactivation by NH_3. Further, the kinetics for double-stranded RNA and DNA viruses should be other than first order because at least two ruptures, one in each of the two nucleotide chains are needed (Thomas, 1956). The NH_3 as produced in sewage sludge by ammonification along with the elevation of pH by the addition of lime and moderate elevation of temperature has promise as a very effective and economical way of inactivating viruses. However, the possible higher resistance of double stranded nucleic acid viruses to NH_3 inactivation must be taken into consideration.

SECTION 5

EFFECTS OF SLUDGE AND COMPOST ON SOIL ENZYMATIC ACTIVITY

INTRODUCTION

Organic soil amendments such as sewage sludge and sewage sludge compost may enhance agricultural productivity by providing nutrients essential for plant growth and by improving soil physical properties. These amendments can provide significant amounts of the macronutrients (N, P, K, S, Ca, and Mg) in addition to such micronutrients as Fe, Mn, and Zn. Improvement in soil physical properties such as increased water retention, aggregation, aeration, permeability, water infiltration, and decreased surface crusting may result when marginal soils are amended with organic waste materials (Epstein et al., 1976).

Although beneficial in many ways, these organic wastes may also contain materials which are detrimental to the soil-plant system. Soil enzymes, important factors in plant nutrient mineralization, may be temporarily or permanently impaired by soil amendments which contain excessive amounts of heavy metals or organic chemicals. A study was initiated to quantitate and compare the effects of various sewage sludges and sludge composts on important soil enzymes such as dehydrogenase, urease, and alkaline phosphatase.

MATERIALS AND METHODS

The undigested, lime stabilized sewage sludge was obtained from Blue Plains Wastewater Treatment Facility, Washington, D.C., and was composted by the Beltsville Aerated Pile Method. Pre-cured compost (PRC) is an intermediate compost product which has not undergone the final curing process (Table 2). A digested sewage sludge that was several years old and contained high levels of organic chemicals and heavy metals was obtained from a lagoon in Philadelphia, PA and composted by the Beltsville Aerated Pile Method (Table 3). A sandy loam soil, belonging to the Evesboro soil series (Typic Quartzipsamment), was obtained from a coniferous site near Beltsville, MD. The soil has a pH of 4.7, contains 0.4% carbon by weight, and has a moisture content of 7.6% at 0.33 bar.

The soil was air-dried, sieved (2mm mesh), and combined with the appropriate amendment at a rate of 0, 3% (67.2 Mg/ha), or 6% (134 Mg/ha). In incubations using lime-stabilized sludge or sludge compost, a CaO treatment was included to determine the effects of lime on enzyme activity. One hundred grams of the mixture was then placed in a wide mouth bottle, adjusted to 0.33 bar moisture content, and allowed to equilibrate at 4°C for one day before starting the incubation. Duplicate sample bottles of each amendment type and rate were attached to a central manifold which provided CO_2-free, humidified air from a bottle source. The airflow was adjusted to a uniform rate of 180 $m^3/h/Mg$ while the temperature of the system was maintained at 25°C during incubation. The exit gases of each bottle were diffused through 2N NaOH to capture CO_2. Each bottle was sampled at seven day intervals and enzyme assays were performed. The dehydrogenase method used was that of L.E. Casida (1977). The urease and alkaline phosphatase (modified) procedures followed were those of P. Nannipieri et al. (1978). The NaOH traps were changed weekly and CO_2 concentration determined by the titrimetric method. Incubations were terminated when enzyme activities leveled, usually after 50 to 70 days.

TABLE 2. CHARACTERISTICS OF BLUE PLAINS SLUDGE AND SLUDGE COMPOSTS

	--------Concentration--------		
Constituent	**Sludge**	**PRC***	**Compost**
Compost			
Percent solids	22	50	60
Percent volatile solids	48	54	42
Kjeldahl Nitrogen (%)	3.4	1.29	1.48
Phosphorus (%)	1.9	1.30	1.67
pH	11.0	-**	7.7
Zinc (mg/kg)	706	486	647
Copper (mg/kg)	284	208	229
Cadmium (mg/kg)	7.3	5.3	6.1
Lead (mg/kg)	-	-	180
Nickel (mg/kg)	-	-	68

* compost after aeration step but before curing step.

**not analyzed

TABLE 3. CHARACTERISTICS OF PHILADELPHIA DIGESTED SLUDGE AND SLUDGE COMPOST

	--------Concentration--------	
Constituent (mg/kg)	**Sludge**	**Compost**
Zinc	5000	2800
Iron	-*	26300
Manganese	640	748
Copper	1400	934
Lead	2100	1370
Cadmium	150	60.8
Nickel	200	93.3
PCB**	30	-
pH	7.3	6.3

* not analyzed

**as Aroclor 1260

RESULTS AND DISCUSSION

In an incubation comparing the effects of the Blue Plains undigested, lime-stabilized sludge to the sludge compost, initial dehydrogenase activity was low for all amendments and rates (Fig. 11). After about one week, activity of the compost mixtures increased in proportion to amendment rate; that is, activity of the 6% compost doubled that of the 3% compost. Peak dehydrogenase activity of the compost mixtures was considerably lower than that of the 3% sludge, indicating that composting decreased the amount of easily degradable material and produced a more "stabilized" product. Throughout the incubation, activity of the 6% sludge was lower than the compost or 3% sludge, probably due to the high pH of this sludge-soil mixture (Table 4). A lower amendment rate or composting of the limed sludge, either of which would result in a pH nearer neutrality, is recommended to prevent inhibition of dehydrogenase activity.

Composting organic wastes by the Beltsville Aerated Pile Method involves curing as part of its stabilization process. An incubation was done to compare the effects of cured versus uncured compost (PRC) on soil enzyme activity. Both alkaline phosphatase activity (Fig. 12) and total CO_2 production (Fig. 13) were greater in the PRC than in cured compost, indicating that PRC is less stable (or contains more biologically degradable material) than cured compost. Phosphatase activity of all amendments was correlated to CO_2 evolution; increasing the amendment rate and/or decreasing the degree of amendment stabilization resulted in an increase in both CO_2 production and phosphatase activity.

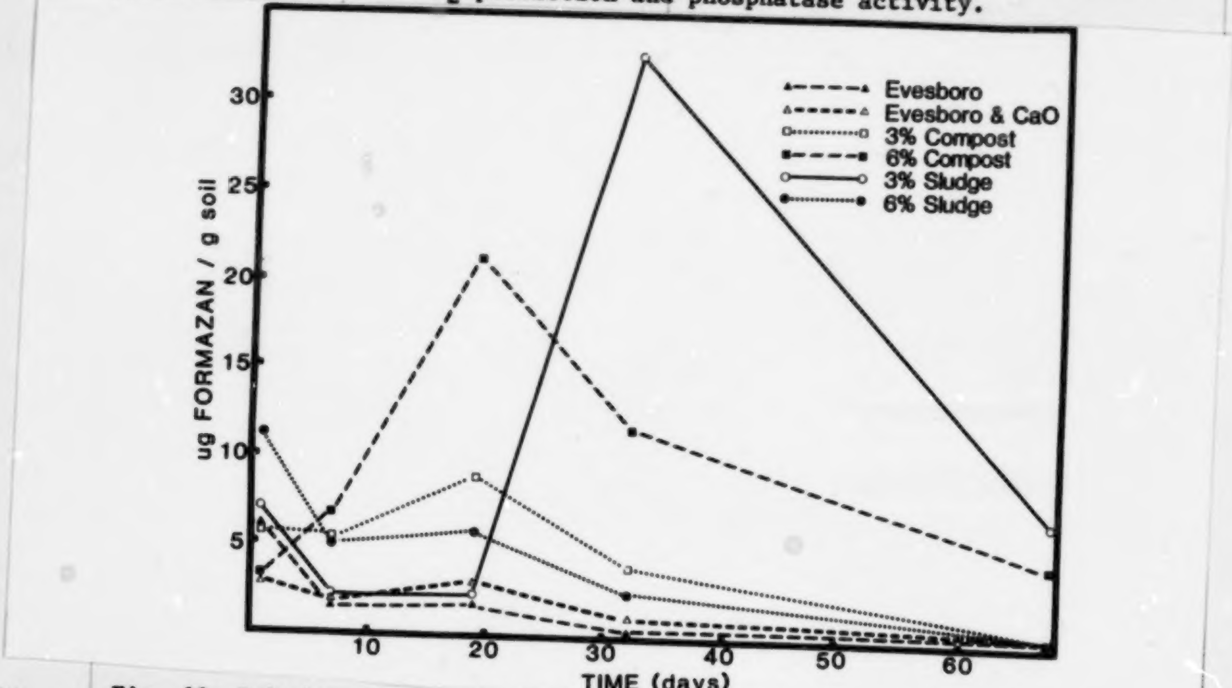

Fig. 11. Dehydrogenase activity in an Evesboro SL soil amended with Blue Plains sludge and sludge compost.

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TABLE 4. pH VALUES AFTER INCUBATION OF EVESBORO SL AMENDED WITH BLUE PLAINS SLUDGE AND SLUDGE COMPOST

Treatment	pH
Evesboro only	4.7
Evesboro + CaO	6.5
Evesboro + 3% compost	7.7
Evesboro + 3% sludge	8.2
Evesboro + 6% compost	7.8
Evesboro + 6% sludge	9.9

Extractable PO_4-P levels of samples taken at the end of the incubation correlated with the relative levels of phosphatase activity. A CaO addition to the soil increased CO_2 production (compared to the control) but decreased phosphatase activity.

A high-metal, high organic chemical sewage sludge and its composted counterpart were tested to determine their effects on soil enzymatic activity. Dehydrogenase activity and rate of CO_2 evolution of the amended soils were closely correlated with respect to amendment type and rate, and no inhibition was apparent (Fig. 14 and 15). If these sludge

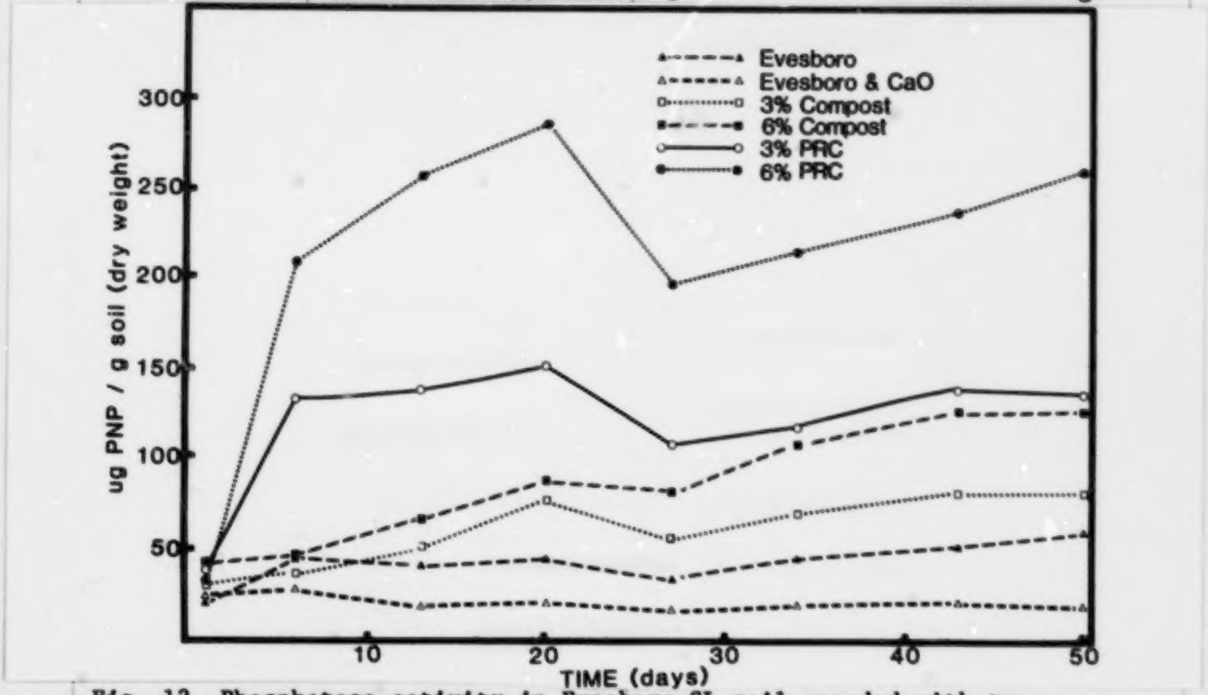

Fig. 12. Phosphatase activity in Evesboro SL soil amended with two undigested lime-stabilized sludge composts.

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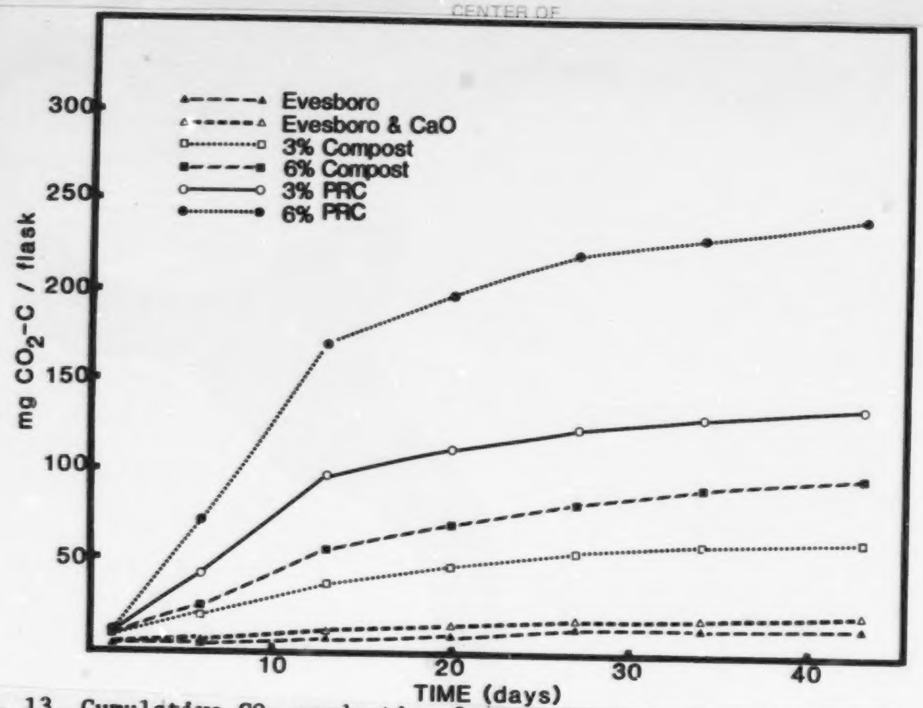

Fig. 13. Cumulative CO_2 production from an Evesboro SL soil amended with two undigested lime-stabilized sludge composts.

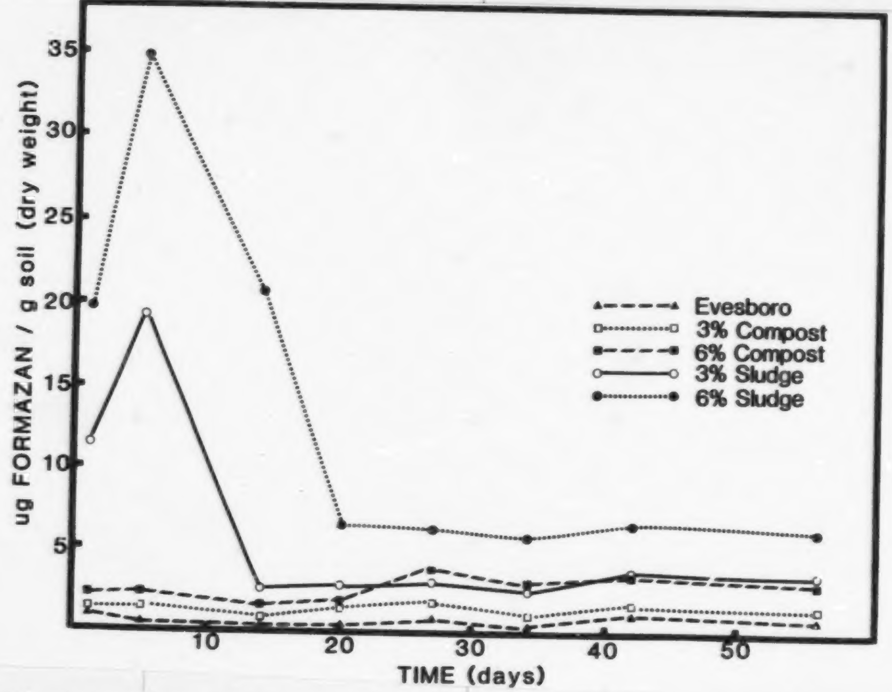

Fig. 14. Dehydrogenase activity in an Evesboro SL soil amended with Philadelphia sludge and sludge compost.

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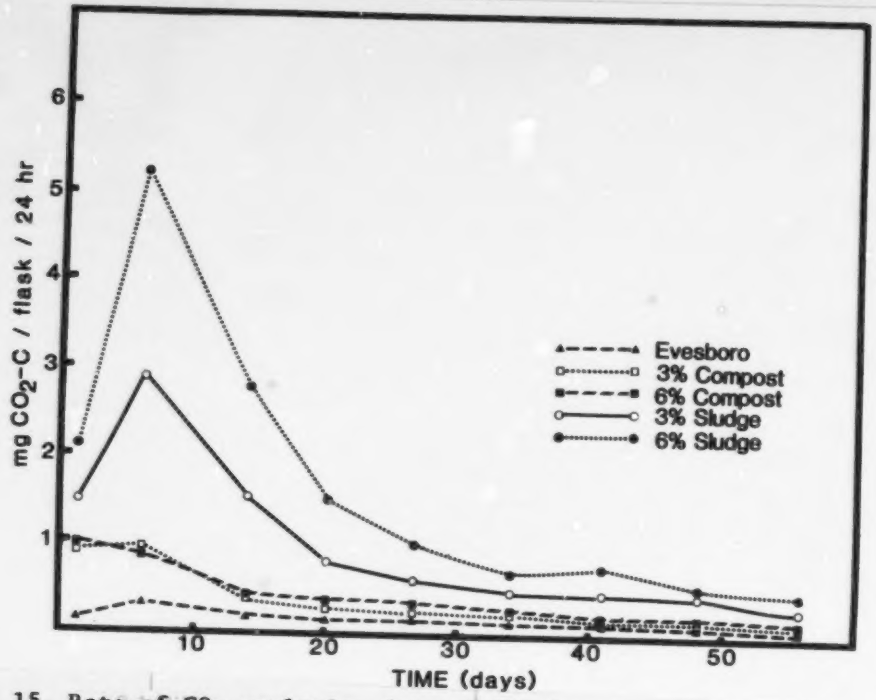

Fig. 15. Rate of CO_2 evolution from an Evesboro SL soil amended with Philadelphia sludge and sludge compost.

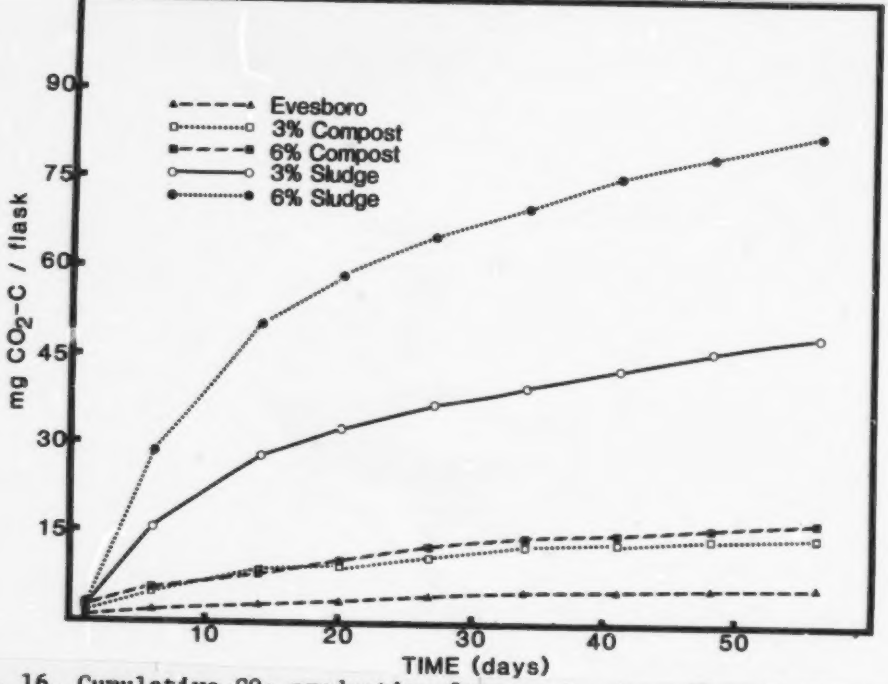

Fig. 16. Cumulative CO_2 production from an Evesboro SL soil amended with Philadelphia sludge and sludge compost.

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and compost amendments were inhibitory, then the increase in amendment rate from 3% to 6% would presumably result in enzyme activity levels something less than double; this was not the case. Although the CO_2 production in this incubation is less than that observed for the same amendment rates in other studies (Fig. 13 and 16), it is likely that the lower activity is a result of the increased stabilization of the lagooned sludge and not inhibition. Composting of the sludge resulted in further stabilization and correspondingly lower activities.

Net urease activity was correlated to amendment type (degree of stabilization) and rate, and showed no apparent inhibition (Fig. 17). Background NH_4 levels were not inversely proportional to net urease activity, a result which might be expected if the feedback mechanism for enzyme synthesis were involved (Fig. 18).

The relative level of heavy metals or organic chemicals therefore may not be a suitable indicator of the biological effects of sewage sludge and sewage sludge composts. Several studies have indicated that inorganic metal salts have inhibiting effects on enzymes, some at very low concentrations (Parr et al., 1983). From these data it appears that, through time, metals are bound or chelated with organic matter and that organic chemicals are partially degraded or neutralized, resulting in little or no inhibitory effect on biological processes such as enzyme activity. The age, or stability, of the material may be an important factor in predicting the effects of such amendments on enzyme activity.

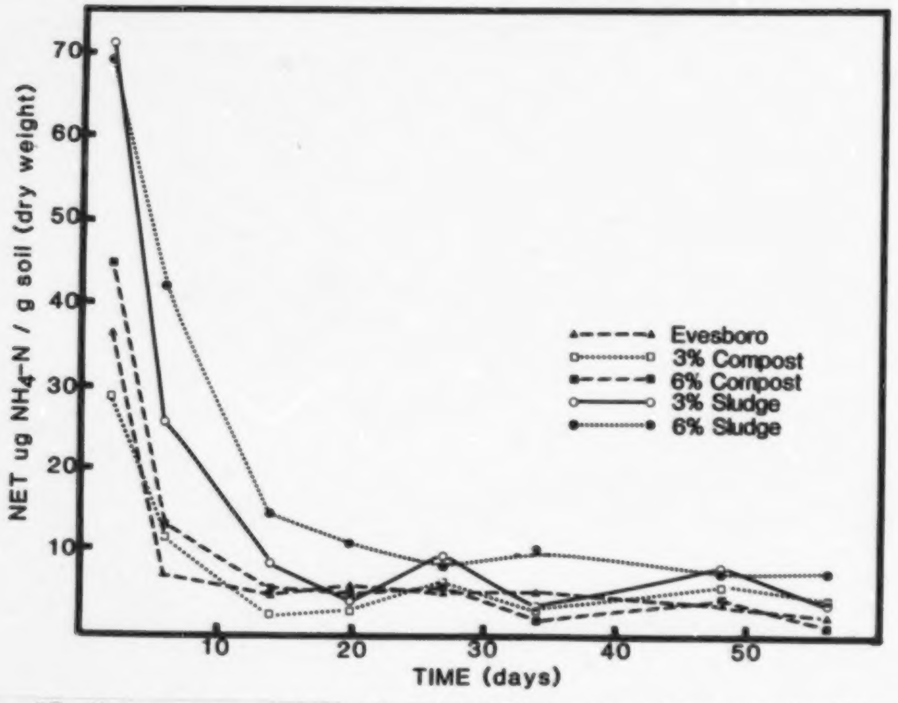

Fig. 17. Net urease activity in an Evesboro SL soil amended with Philadelphia sludge and sludge compost.

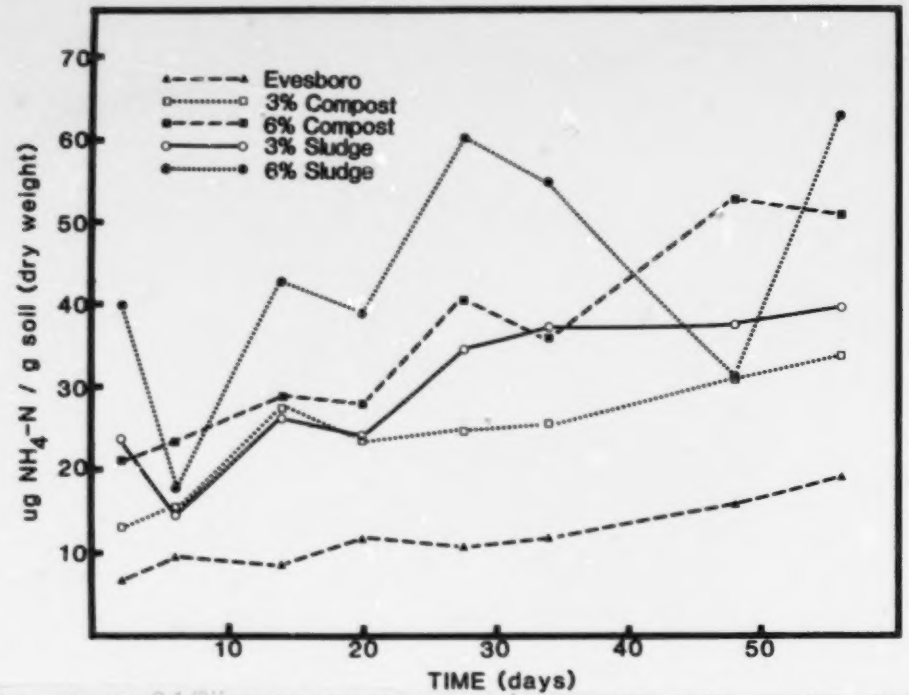

Fig. 18. Background NH_4 levels in an Evesboro SL soil amended with Philadelphia sludge and sludge compost.

CONCLUSIONS

In evaluating the effects of sewage sludge and sewage sludge compost on soil enzyme activity, it was found that:

1) amending soil with highly-limed sludge inhibited enzyme activity probably due to the resultant high pH of the mixture; compost amendments from the same sludge were not inhibitory;

2) stabilization level of the organic amendment was correlated negatively with enzyme activity;

3) high concentrations of heavy metals and chlorinated organic materials in a sludge and sludge compost appeared to have no inhibitory effect on soil enzyme activity, probably because of the age or the highly-stabilized nature of the sludge and sludge compost.

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SECTION 6

W-124 COOPERATIVE STUDY - EFFECTS OF SEWAGE SLUDGE APPLICATIONS ON NITROGEN MINERALIZATION AND PHOSPHORUS MOBILITY IN SOILS

INTRODUCTION

Sewage sludges contain a wide range of essential plant nutrients. Recycling these nutrients on agricultural land can serve to augment commercial fertilizer sources and thereby conserve the energy used in their production.

Sufficient data are available to show that the indiscriminate use of large amounts of sewage sludges on lands may damage crops, impair their quality, and serve as a source of surface and groundwater pollution. Also we know that modest amounts of sewage sludges from a number of sources applied to agricultural land serve as a valuable source of plant nutrients and cause no adverse environmental effects. However, most of the information accumulated on both possible detrimental and beneficial effects is more or less site specific, and because of the non-uniformity of methodology employed it is impossible to generalize regarding effects of sludge applications over a broad range of sludge characteristics, crop and soil types, and climates. This regional study proposed will standardize the methodology used among the cooperating states in the North Central and Western region, and thereby develop the data needed to predict long term effects of sludge applications under a wide variety of conditions.

In 1978, the W-124 Regional Committee formulated a cooperative research project on land application of sewage sludge in order to answer the questions concerning the effect of soil type and climate on N mineralization, P mobility, and heavy metal availability in sewage sludge amended soils. Two sets of field plots were established at Beltsville, one to evaluate heavy metal availability and the other to evaluate N mineralization and P mobility in the soil profile. The following section will discuss the latter study. The results will ultimately be integrated into the data from the larger study.

MATERIALS AND METHODS

Plots were located in a Sunnyside fine sandy loam (Typic Hapludult) and were 7 by 7 m with a 3 m border. Prior to amending the plots with sludge and fertilizer, deep core samples were taken with a tractor-mounted Giddings soil probe. Samples were taken to varying depths depending upon obstructions, divided into 15 cm increments for samples near the surface, and 30 or 60 cm increments for deeper samples. Samples were later dried, crushed, and analyzed for total N and P content.

The sludge used in the study, a digested sludge from Chicago, Ill., was distributed to the various cooperators by the USEPA, Cincinnati. The fertilizer amendments were NPK and PK, added at rates of N, P, and K equally 89.6, 44.8, and 44.8 kg/ha, respectively. The sludge amendments were 20 Mg/ha annually and 100 Mg/ha once in the first year. In the third year the sludge plots were split and one-half was fertilized with nitrogen at 89.6 kg/ha. The amendments (sludge or fertilizer) were spread on the plots by hand and the plots were rototilled. In 1978 and 1979, the barley (Hordeum vulgare L., Barsoy variety) was spread by hand and raked into the surface soil. Poor germination was experienced the second year which lead to the decision of drilling in the seed in the remaining years of the study. The barley was seeded at a rate of 21.7 kg/ha in rows 18 cm apart. To prepare the seed bed prior to drilling, the plots were disked prior to drilling.

Spraying for weeds was performed in the fall at tillering time using 2, 4-D (4.0 g per 1, 80 1 per ha). The barley was harvested in June by taking all plants in a 1 m^2 area (using 7 rows). The plants were weighed in the field, later dried at 70°C, reweighed, the heads removed, and the heads (minus chaff) and straw ground through a 2 mm mesh using a stainless steel mill. The tissue was analyzed for N and P content after digestion in a block digestor (AWPA, 1980), and for heavy metals according to methods listed in Chaney et al., (1976).

Ten soil samples (2.5 cm diam, 15 cm deep) were taken from each plot on an annual basis after harvest made into a compost, were extracted with 0.5 M $NaHCO_3$ (5 g in 50 ml) for 16 hours, filtered, and analyzed for ortho-phosphate using Antoanalyzer methods (AWPA, 1980). A 3:1 w/w ratio mixture of 0.01 M $CaCl_2$ to soil sample were made for pH determination.

RESULTS AND DISCUSSION

The data reported includes the 1979 to 1983 individual harvests as well and the cumulative data (Table 5 to 8). In general the data indicated that 20 Mg/ha annual application rate resulted in yields similar to those from the recommended fertilizer application rate, but slightly more N was taken up by the plants growing on plots receiving sludge. The 20 Mg/ha annual application resulted in a greater cumulative yield and N uptake than the single 100 Mg/ha application added to the plots in 1978. The application of N on the split plots in 1981 resulted in larger but not significant yield increases and N uptake increases in most instances (Table 5 and 6). These increases appeared to be greater on the 100 Mg/ha plots versus the 20 Mg/ha plots, probably because the amount of mineralizable N in the 100 Mg/ha plots in any given year was less than in the plots receiving 20 mt/ha annually.

Phosphorus uptake was greater in the first and second year from the 100 Mg/ha than the 20 Mg/ha plots, after which the uptake for the third through the fifth year was the same for both treatments (Table 7). The

TABLE 5. ANNUAL AND CUMULATIVE YIELDS IN Mg/ha FOR BARLEY FROM W-124 PLOTS.

Treatment	1979	1980	1981	1982	1983	Cumulative
	TOTAL YIELD (Mg/ha)					
PK	5.1 A *	3.1 B	4.2 B	4.6 C	3.0 C	20.4
NPK	6.4 A	4.2 A	10.4 A	13.2 AB	8.8 AB	43.0
20 Mg/ha	6.6 A	2.4 B	10.1 A	14.8 AB	8.1 AB	42.0
20 Mg/ha+N			9.6 A	15.5 A	10.6 A	35.7
100 Mg/ha	6.6 A	4.5 A	8.5 A	8.2 BC	5.5 BC	33.3
100 Mg/ha+N			9.0 A	11.4 ABC	8.2 AB	28.2

* Numbers in columns followed by the same letter are not significantly different at the 5% level.

TABLE 6. ANNUAL AND CUMULATIVE TOTAL N UPTAKE IN kg/ha BY BARLEY FROM W-124 PLOTS.

Treatment	1979	1980	1981	1982	1983	Cumulative
	TOTAL NITROGEN UPTAKE (kg/ha)					
PK	55.7 B *	26.2 B	45.0 B	26.8 B	30.5 C	184.2
NPK	73.5 AB	34.1 AB	89.5 A	33.3 B	95.6 AB	326.0
20 Mg/ha	76.5 AB	26.1 B	102.0 A	52.6 AB	99.9 AB	357.1
20 Mg/ha+N			86.0 A	78.0 A	145.0 A	309.0
100 Mg/ha	95.1 A	46.0 A	79.9 A	37.8 AB	62.9 BC	321.7
100 Mg/ha+N			84.0 A	52.1 AB	87.7 ABC	223.8

* Numbers in columns followed by the same letter are not significantly different at the 5% level.

TABLE 7. ANNUAL AND CUMULATIVE TOTAL P UPTAKE IN kg/ha BY BARLEY FROM W-124 PLOTS.

Treatment	1979	1980	1981	1982	1983	Cumulative
		TOTAL PHOSPHORUS UPTAKE (kg/ha)				
PK		7.0 C	13.1 B	27.5 B	7.0 C	54.6
NPK		8.7 B	22.6 B	35.6 AB	15.3 ABC	82.2
20 Mg/ha		6.8 C	21.0 A	47.0 AB	16.7 AB	91.5
20 Mg/ha+N			20.7 B	33.5 A	21.8 A	73.0
100 Mg/ha		10.2 A	47.4 A	38.3 AB	12.9 BC	108.8
100 Mg/ha+N			60.8 A	38.2 AB	15.5 ABC	114.5

* Numbers in columns followed by the same letter are not significantly different at the 5% level.

TABLE 8. MEANS EXTRACTABLE PHOSPHORUS LEVELS IN SURFACE SOIL SAMPLES FROM PLOTS RECEIVING VARIOUS TREATMENTS IN THE W-124 COOPERATIVE STUDY FROM 1979 THROUGH 1983.

Treatment	mg P/Kg soil, dry weight basis 1979	1980	1981	1982	1983
PK	42.5	43.6	41.2	48.1	49.6
NPK	36.2	38.7	35.3	42.4	55.0
20 Mg/ha	46.5	48.9	57.4	138.0	130.2
20 Mg/ha+N	-	-	54.7	70.2 *	141.0
100 Mg/ha	116.0	118.0	95.9	95.5	145.0
100 Mg/ha+N	-	-	83.0	93.9	143.0

* - range = 62.4 - 135.1

extractable P levels were much higher in the 100 Mg/ha plots than in the 20 Mg/ha plots and remained higher through 1982. In 1983, levels of extractable P increased again significantly for unknown reasons. The annual 20 Mg/ha application resulted in annual increases in extractable P but for some reason, in 1982 a larger than normal increase in extractable P was seen (Table 8). The split plot amended with N had a lower mean value in 1982 but also had sizeable range of values. A "t" test was performed comparing the P uptake by barley grown on the 20 Mg/ha (N split included) in 1982 with 1983 and the test indicated that there was no significant difference in extractable P levels in 1982 and 1983. A similar test of the 100 Mg/ha data indicated that in 1982 the extractable P was significantly lower than in 1983. Again, the reason for the increase in extractable P uptake in 1983 on the 100 Mg/ha plots was unknown.

Soil samples were taken with depth after the harvest in 1983 and compared with soil samples taken from the plots in 1978 prior to sludge application. The total N and P contents of the soil were compared with, in some cases, samples from the same plot to determine the migration of N and P due to the application of sludge. Migration of N was detected to the deepest sampling depth in all N and sludge fertilized plots (Table 9). The increase in total N in the surface soil of the PK plots may be due to runoff from adjacent plots. Phosphorus migration down to 60 cm was found in plots receiving sludge application. The sampling in 1983 was hampered because of dry soil conditions which allowed sampling only to a depth of 180 cm in some plots.

CONCLUSION

The major purpose of this portion of the W-124 study was to determine the mineralization and mobility of N and P from sewage sludge. The data indicate that N movement down the soil profile occurred in the sludge amended plots, especially in the split plots which received the additional fertilizer (Table 9). Although no significant difference in crop uptake

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TABLE 9. TOTAL N AND P CONTENT (MEAN mg/kg, DRY WEIGHT) IN SOIL SAMPLES TAKEN WITH DEPTH IN PLOTS SAMPLED IN 1978 BEFORE SLUDGE AND FERTILIZER APPLICATION AND IN 1983.

Plot Treatment	Depth of Samples (cm)	Total N		Total P	
		1978	1983	1978	1983
PK	0-15	682. *	943.	363.	397.
	15-30	328.	437.	238.	241.
	30-60	121.	250.	143.	160.
NPK	0-15	737.	977.	352.	393.
	15-30	367.	550.	247.	223.
	30-60	126.	233.	149.	157.
	60-90	76.	220.	102.	100.
	90-120	72.	130.	109.	100.
20 Mg/ha	0-15	546.	1420.	361.	760.
	15-30	226.	697.	187.	537.
	30-60	134.	273.	137.	150.
	60-90	77.	250.	118.	180.
20 Mg/ha+N	0-15	546.	1170.	361.	757.
	15-30	226.	893.	187.	460.
	30-60	134. **	275.	137.	165.
	60-90	77. **	205.	118.	135.
	90-120	73. †	230.	146.	140.
	120-150	95. †	290.	126.	190.
	150-180	77. †	170.	128.	140.
100 Mg/ha	0-15	631. **	1320.	342	933.
	15-30	248. **	560.	190.	310.
	30-60	108. **	277.	131.	177.
100 Mg/ha+N	0-15	631. **	1160.	342.	780.
	15-30	248. **	365.	190.	230.
	30-60	108. **	205.	131.	155.
	60-90	108. †	240.	169.	150.

* All values are means of composite samples from 3 replicate plots except where otherwise indicated.

** Values are means of composite samples from the same 2 replicate plots sampled in 1978 and 1983.

† Values are a single composite from the same plot sampled in 1978 and 1983.

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was recorded in most instances (Table 6) the soils profiles in the sludge amended plots had markedly more N in the deep samples. Therefore, the amount of available N from 20 Mg/ha sludge application is more than barley can utilize in a single growing season.

The mobility of P from sewage sludge amendments has received substantially less attention than the mobility of N. Phosphorus movement into the subsoil in fields amended with composted sewage sludge (Darmody et al. (1983) and sewage effluent (Latterell et al., 1982) has been reported. The movement does not appear to be as extensive as phosphorus leaching in soils amended with manure (Johnston, 1981).

The P form that is moving may be organic P or finely divided soil particles that migrate with infiltrating water (Fordham and Schwertmann, 1977). the depth of migration cannot be predicted readily because several factors play a role in P adsorption (Sikora and Corey, 1976).

The movement of sludge N through the soil profile is of concern and can be controlled with accurate N mineralization predictions for both sludge and soil. Most states allowing sewage sludge application to agricultural land require matching crop N needs to application rate. The movement of P does not pose a problem and may even be beneficial. The depth of P movement is within the root zone and below the level of incorporation. Therefore, plants should benefit from the deeper soil horizons containing P.

SECTION 7

EFFECT OF TEMPERATURE AND AERATION ON THE COMPOSTING PROCESS

INTRODUCTION

Composting as a method of drying and stabilizing sewage sludge has gained popularity in the United States. Fig. 19 indicates the sewage sludge composting facilities presently in operation in the U.S. with the total number equalling 61 (Willson and Dalmat, 1983). Although windrow composting of digested sludge has been practiced successfully in Los Angeles for nearly twenty years, the present trend is for municipalities to use the static pile aeration method for composting sewage sludge. Advantages of the static pile method over the windrow method include composting more sludge per unit area, controlling odors with the odor filter pile and/or blanket of composted material, and providing sufficiently high temperatures through the entire mixture to kill pathogenic organisms (Willson et al., 1980).

Composting is a process that is self-starting, self-heating, and in certain circumstances, self-limiting (Finstein et al., 1983). In general, the variables which control or affect composting are the quality of the starting materials, moisture, aeration, pH, and temperature.

Composting is an aerobic decomposition process which produces heat that is conserved in the mass according to such physical factors as heat capacity, radiation, and convection, (Haug, 1980). Biological degradation ceases when the most limiting essential nutrient or element is exhausted. After organic or carbonaceous material, nitrogen is the next major nutrient required by microorganisms followed by phosphorus, potassium, magnesium, sulphur, calcium, and several trace metals (Gray et al., 1971b). The cooling of compost piles is most often caused by depletion of the carbonaceous energy supply. The C:N ratio affects the composting process signficantly and a ratio of 25-50:1 is desirable. If the C:N ratio is higher, then the microorganisms must go through several cycles oxidizing excess carbon until the ratio of stabilized biomass (10:1) is reached (Gray et al., 1971b; Poincelot, 1975). Nitrogen limitation normally will occur in this system before all of the degradable organic material is decomposed. A low C:N ratio (10:1 or less) will result in N loss either by leaching or volatilization from the compost which may reduce the value of the final compost as a fertilizer. In composting of human wastes or sewage sludge, the C:N ratio is normally adjusted from 10:1 to 30:1 ratio by addition of carbonaceous materials to make the process more efficient, while in the composting of bark, nitrogen is added to reduce the inherently high C:N ratio to stimulate the process. As

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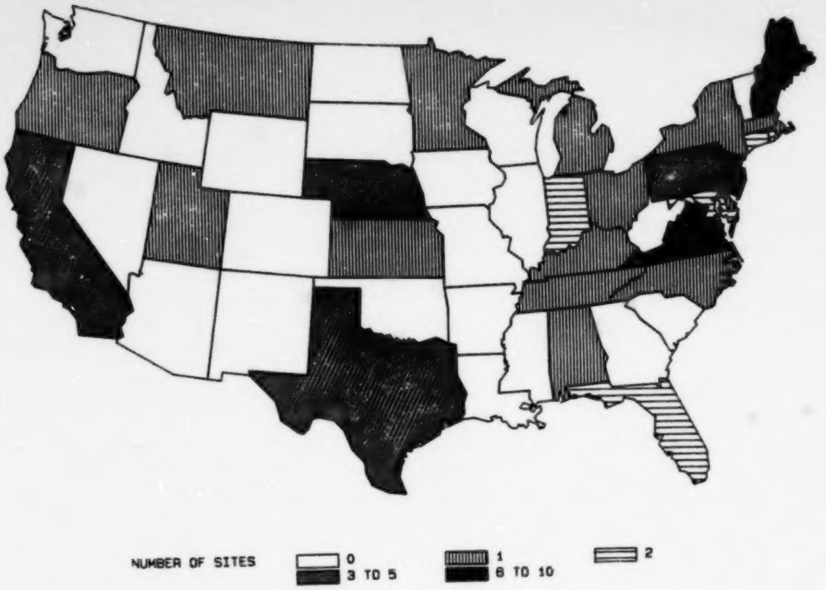

Fig. 19. Number of sewage sludge composting facilities operating in each state of the United States in 1983 (from Hornick et al., 1983).

Finstein and Morris (1975) pointed out, composting under a nitrogen deficiency may be desirable to slow the composting process when O_2 replenishment is limited. The example given was a leaf composting system where, with addition of nitrogen, decomposition would be rapid and without frequent turning of the piles, anaerobiosis and odor production would take place.

Moisture is necessary for biological activity but extremes in moisture content in composting systems will lead to undesirable effects on the process. Snell (1957) showed that oxygen uptake during composting at moisture contents below 30% was approximately one sixth that at 55% moisture which was considered optimum. Excess moisture (>70%) does not limit biological activity per se, but does lead to less efficient degradation in the form of anaerobic decomposition. Oxygen diffusion in water is several times less than in air and in compost piles containing high moisture contents, the majority of pores are filled with water which limits the replenishment of O_2.

In the process of removing water from composts, heat is concomitantly removed. Vaporization is the dominant heat removal mechanism, removing nearly nine times more heat than convection (Macgregor et al., 1981). Evaporation can be accelerated by turning piles or, in the aerated pile by increasing the aeration rate. Therefore, the control of temperature is best accomplished through control of vaporization or moisture removal.

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The preponderance of information on the effects of temperature on composting indicate that optimum decomposition takes place between 55 and 60°C. Other temperature peaks below 50°C have been recorded (Snell, 1957), but the composting process is most often associated with temperatures above 50°C. Suler and Finstein (1977) and Macgregor et al. (1981) indicated that maximum CO_2 and heat production occurred at temperatures near 55°C. The reason for optimum decomposition in this range is microbial population diversity (Finstein and Morris, 1975). At higher temperatures, fungi are completely inhibited and bacteria and actinomycete populations are reduced to thermotolerant species. The reduced fungal population itself would decrease the decomposition of several substrates generally difficult to degrade by other microorganisms such as cellulose. However, high temperature composting is more commonly seen than low or moderate temperature composting because of the self-heating concept and, in instances where pathogenic organism kill is of high priority in the composting system, high temperatures are desirable to ensure a pathogen-free product.

Very low or very high pH's slow down the initiation of the compost process (Sikora et al., 1983). The effect of extreme pH on the composting process is directly related to pH effects on microbial activity. The tendency for final composts to be neutral (pH 6 to 8) underlines one of the benefits of composts as a suitable soil amendment in addition to a source of organic material or nutrients.

Several studies have been conducted to evaluate the decompositon of organic wastes during composting and the experimental units have been piles or windrows (Willson and Walker, 1973), externally heated bench composters (Bagstam et al., 1974; Suler and Finstein, 1977; Willson, 1971; Ashbolt and Line, 1982), and self-heating or adiabatic composters used for laboratory studies (Nell and Wiechers, 1978; Mote and Griffis, 1979; Norman et al., 1941; Walker and Harrison, 1960). Large piles or windrows preserve the self-heating concept of composting and the microbial succession that occurs during the heating and cooling trends but mass balances on constituents are difficult to obtain in such open air systems. Bench composters are enclosed systems that are externally heated according to predetermined program either to match heating cycles from open air piles or to evaluate decomposition at a preset temperature range. Mass balances are obtained but researchers occasionally criticize the time-temperature programs and microbial succession for being artificial. The adiabatic composter preserves the self-heating concept and is enclosed in order that mass balances can be calculated.

To achieve the self-heating concept, a laboratory composter is designed to minimize heat loss by insulating the organic material with a medium such as water, oil, or air. A temperature comparison mechanism senses the temperature of the decomposing organic material and adjusts the temperature of the insulating medium to within 0.1 to 0.2°C below that of the mass. A system with this design results in temperature decreases or increases according to the decomposition rate and heat output of the organic material. Compost piles perform similarly and therefore these self-heating and, similarly, cooling systems are excellent tools for studying the composting process.

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The temperature comparison mechanisms differ primarily in their temperature sensing devices. Mote and Griffis (1979) used a system of copper-constantin thermocouples and a comparator circuitry to measure the temperature of the mass and subsequently heat the insulating bath to just below the temperature of the mass. Walker and Harrison (1960) and Norman et al. (1941) also used thermocouples and galvanometer amplifier with a light beam that, when deflected onto a photoconductive cell, actuated the insulating water bath. Nell and Wiechers (1978) used an electronic control system consisting of closely matched transitors.

Nearly all laboratory composters pass humidified air through the compost mass to maintain aerobic conditions without drying out the mass. The Mote and Griffis (1979) design included on O_2 replenishment system which was actuated when the volume of the head space over the mass decreased a set amount.

A laboratory composter which utilizes thermistors as temperature sensing devices and an aeration system which permits diffusion of air into the mass was designed. Data obtained with this apparatus from the composting of a sewage sludge-woodchip mixture include the interaction between CO_2 and temperature and CO_2 and NH_3 loss which, will provide insight into the possible control of the composting process to obtain a more stabilized product with minimal loss of N.

MATERIALS AND METHODS

Laboratory Composter Design

The self-heating laboratory composter is comprised of a covered, double-walled, insulated tank, an air-tight cylinder, an inner screen mesh cylinder which holds the organic material, a heater-circulator, and a differential temperature control system (Fig. 20). The inner wall of the tank is made of 0.31 cm aluminum sheet, the outer wall 1.58 cm plywood, and are separated by 2.54 cm styrofoam insulation. The internal dimensions of the tank are 59 cm wide by 79 cm long by 46 cm high. The cover consists of both stationary and removeable sections which are made of 0.31 cm aluminum sheet. Holes were drilled in the stationary section for passage of the thermistors and the heater-circulator. Various ports in the tank for water and air passage were made with 1.2 cm plastic pipe threaded into a 1.0 cm thick aluminum plate riveted to the inner tank.

The air-tight cylinder in which the mesh cylinder is placed is 30 cm by 25 cm in diameter and is made of 0.08 cm stainless steel. One end plate is 0.08 cm stainless steel and is soldered into place. The removeable end plate is 0.15 cm stainless steel which is attached with wing nuts to bolts soldered to L-brackets attached to the outside of the cylinder. A neoprene gasket (.48cm) placed between the end plate and cylinder lip provides an air-tight seal. Ports for air flow and condensate removal are 0.63 cm stainless steel tube fittings soldered to the cylinder walls or end plates. At the center of the removeable plate is a 0.95 cm male tube fitting for passage of the thermistor and thermocouple. A brace attached to the wall of the tank holds the cylinder in place.

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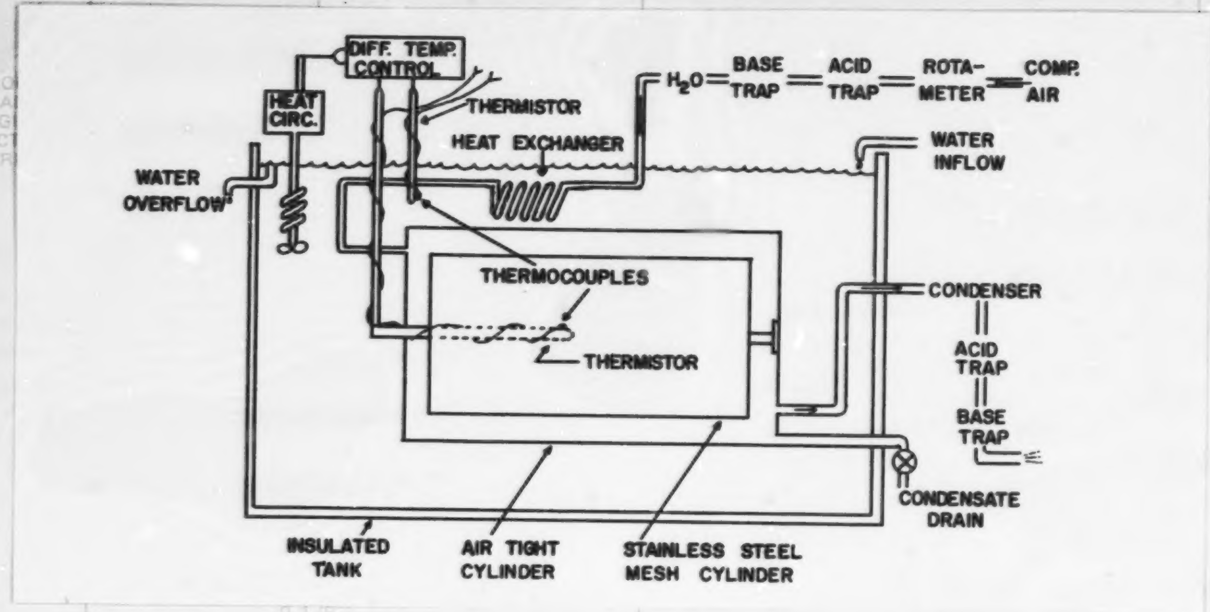
DIFF. TEMP. CONTROL
HEAT CIRC.
THERMISTOR
HEAT EXCHANGER
H_2O
BASE TRAP
ACID TRAP
ROTA-METER
COMP. AIR
WATER INFLOW
WATER OVERFLOW
THERMOCOUPLES
THERMISTOR
CONDENSER
ACID TRAP
BASE TRAP
CONDENSATE DRAIN
INSULATED TANK
AIR TIGHT CYLINDER
STAINLESS STEEL MESH CYLINDER

Fig. 20. Schematic diagram of self-heating laboratory composter.

The inner cylinder which contains the organic material is 25 cm in height and 20 cm in diameter and is made of 12 mesh stainless steel cloth with 0.15 cm thick stainless steel end plates. For the high aeration studies, a 10 cm cylinder was built. One end plate is spot welded to the wire mesh and has a tube fitting which positions the cylinder inside the air-tight outer cylinder. The other end plate is removeable and has a 0.95 cm diameter hole to allow passage of the thermistor and thermocouple. Bolts soldered to L-brackets which are riveted to the cloth are used to fasten the removeable endplate.

Compressed air, regulated with a rotameter, passes through 1N H_2SO_4 to remove NH_3, 2N NaOH to remove CO_2, water for humidification, and a heat exchanger submerged in the water bath before entering the cylinder. All connections are made with .95 mm (I.D.) tygon tubing. As opposed to forcing air through the mass, air passes around the organic material which allows for diffusion to occur. After leaving the cylinder and the tank, the air subsequently passes through a condenser flask, 1N H_2SO_4 trap to capture NH_3, and a 2N NaOH trap to capture CO_2.

The electronic control system senses the temperature in the compost and water with thermistors. The precision, interchangeable thermistors which were sealed inside stainless steel sheathing that extends from the tip of the thermistor to outside the bath are basically semiconductors which exhibit rapid and large changes in resistance with small changes in temperature. The sensor bridge consists of thermistor elements TH1 and

TH2, fixed resistors R2 and R3, and a dial potentiometer, R1A-R1B (Fig. A-1). The 50 ohm potentiometer allows for a maximum temperature differential between the compost and bath of ± 5°C. The fixed resistors, R2 and R3, were selected to maintain bridge balance over this temperature range. Operational amplifier, U1, detects any imbalance in the bridge and drives the Q1 transistor which, in turn, supplied current through R6 to the emitter portion of the optoisolator, U2 (Table A-1). The detector portion of U2 drives Q2 which controls the gate current of the triac, CR 5, turning the heater on and off. The specifications of all components are listed in Table A-1.

One modification was made to the self-heating laboratory composter for some of the studies. The cylinder which contains the mixture was made smaller (10 cm versus 20 cm in diameter) because the smaller mass allowed control of the temperature by the aeration apparatus. The volume of the 10 cm was 25% that of the 20 cm cylinder.

Raw, highly limed, filter cake sludge from the Blue Pains Waste Water Treatment plant (Table 10) was mixed with woodchips in a ratio of 1:1.8 (v/v), and composted. The materials were hand mixed, placed into the mesh cylinder, and weighed. The mesh cylinder was then placed in the outer cylinder, the cylinder sealed air-tight, the thermistor and thermocouple inserted through the port, the tubing was connected to the fittings, and the cylinder submerged in the water bath. Aeration was controlled at either preset airflow rates or according to the demand necessary to hold to the temperature desired. The temperature differential between the sludge-woodchip mix and the bath was set at 0.5°C.

Traps were changed daily, and samples of the traps refrigerated until analysis. Carbon dioxide analysis was done by direct titration using 0.833N H_2SO_4. The Technicon Autoanalyzer II method (APHA, 1980) was used for NH_3 analysis. Samples were heated for 24 h at 105°C for moisture determinations, and 550°C for 24 h after oven drying for

TABLE 10. COMPONENTS OF RAW, LIMED-SLUDGE FROM THE BLUE PLAINS WASTE WATER TREATMENT PLANT IN WASHINGTON, D.C.

Component	Units
Lime, % of solids	22
Ferric chloride, % of solids	6
Primary/secondary ratio	1.5
Percent solids	22
Percent volatile solids	55
Total Kjeldahl N, %	3.4
Total phosphorus, %	1.5
Zinc, mg/kg	980
Copper, mg/kg	420
Cadmium, mg/kg	10
Lead	425
pH	11.0

volatile solids determination. Total wet weight loss was calculated from a single weighing before and after composting, and total dry weight, volatile solids, and Kjeldahl N are means of six replicates. Temperatures of the bath and sludge-woodchip mix were recorded continuously using a multipoint recorder.

Microbiological analyses of the sludge-woodchip mixture before and after composting was performed in the temperature demand experiment to determine changes in the type and number of microorganisms during composting. Visual observation of composted mixtures in several other laboratory composter studies indicated that the composted sludge-woodchip mixture which reached temperatures in excess of 65°C was reddish-brown in color and contained little mycelial growth. In mixtures which peaked at lower temperatures, the color was grey because of mycelial growth which was especially abundant in the composts with peak temperatures below 60°C.

Microbial analyses involved preparing extracts of sludge-woodchips mixtures by mixing 50g samples with 450 ml of sterile water for 1.0 h. Sterile dilutions made with sterile water were made from the extract and plated. Bacteria were grown in tryptic soy broth at 45°C for 2 d. An MPN procedure using microtiter plates was used for bacterial number determination. Fungi were grown on Oxgall media (Millner et al., 1980) containing gentomycin and incubated at 45°C for 3 d. Actinomycetes were grown on a Rhodococcus medium (Rowbotham and Cross, 1977) containing cycloheximide and incubated at 50°C for 3 to 4 d.

RESULTS

No Temperature Limitations

The results of a composting run in which the 20 cm diameter cylinder was used, no temperature limitations imposed and a 110 m^3/h/Mg aeration rate are reported in Fig. 21. A maximum temperature of 82°C was achieved on day 4 and temperatures decreased slowly to 56°C at day 33. A secondary temperature peak on day 38 after which the temperatures declined. The data are similar to those reported as typical field temperature curves by Willson et al. (1980).

The rise in temperature was the result of organic matter decomposition as indicated by the CO_2 evolution data (Fig. 21). Carbon dioxide evolution peaked and then declined when the temperature reached their maximum which indicated the possibility that the high temperature had an adverse effect on microbial decomposition. Depletion of substrate also may have resulted in CO_2 decline but secondary CO_2 peaks are indicative of substrate still available. Carlyle and Norman (1941) observed in their studies of plant material decomposition that only a small portion of total plant constituents is degraded at temperatures near 70°C. After these respective temperature increases, CO_2 evolution generally declined along with temperatures.

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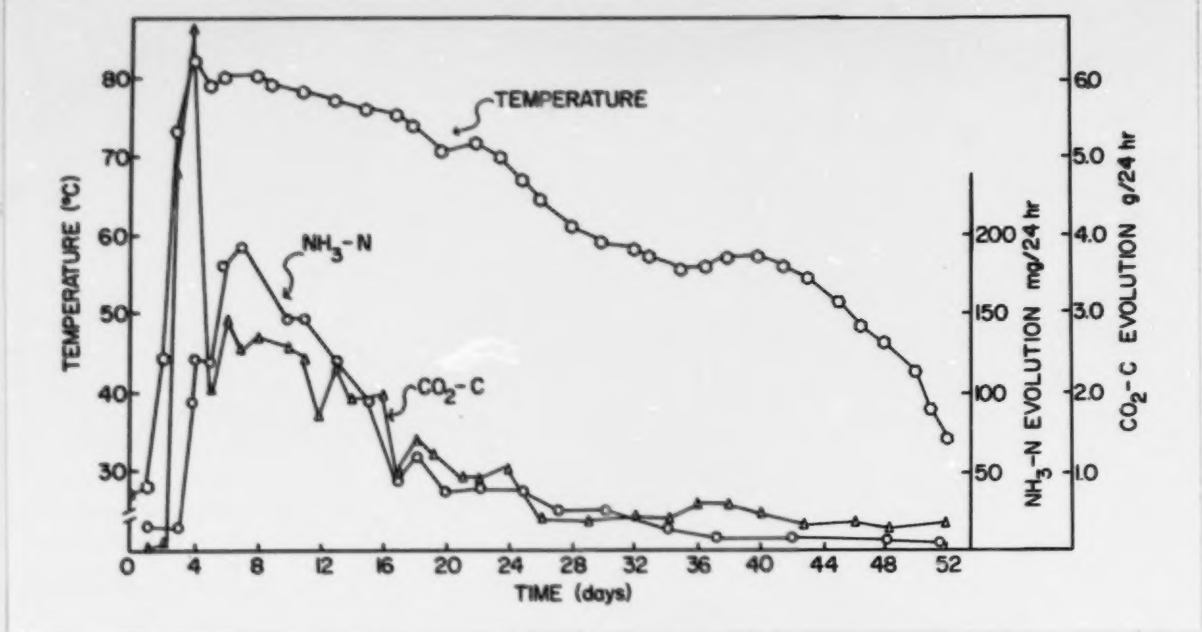

Fig. 21. Temperature, CO_2-C, and NH_3-N loss recorded from laboratory composter study using aeration rate similar to that recommended by Willson et al., (1980).

Ammonia loss from the sludge-woodchip mixture reported in Fig. 21 as evolution per 24 h was the summation of that found in the condensate trapped outside the tank plus that found in the acid traps. Generally, twice as much NH_3-N was found in the condensate as in the acid trap, presumably due to the high solubility of NH_3 in water (Handbook of Chem. and Physics, 1971). Increases in NH_3-N loss followed increases in CO_2 evolution by approximately 2 days and at times when CO_2 evolution decreased (Fig. 21).

As expected, dry weight and volatile solid losses were related (Table 11) and the dry weight loss was near that recorded in a field study conducted using the same sludge and woodchips (Sikora et al., 1981).

A 55° Temperature Limitation

Laboratory studies were then undertaken to determine the effects of controlling the temperatures to near the range reported to be optimum for decomposition (Suler and Finstein, 1977; Wiley, 1957). A temperature limit of 55°C was imposed upon the insulating water bath, thereby not allowing the composting organic material to reach 60°C. Another laboratory composter without temperature limits and an equal aeration rate of 900 m^3/h/dry Mg was used as a control. The control composter had the typical major and secondary peaks followed by cooling to near ambient temperature (Fig. 22). Interestingly, the 55°C composter heated and cooled at approximately the same time. If the correlation was not

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TABLE 11. CARBON, NITROGEN, AND MOISTURE LOSSES DURING COMPOSTING OF RAW, LIMED SEWAGE SLUDGE-WOODCHIP MIXTURES UNDER VARIOUS AERATION TEMPERATURE CONDITIONS.

Aeration-Temperature Condition	--- Materials Losses (%)---				
	Wet Weight	Dry Weight	Total N	CO_2-C*	Final % Moist.
Bath Controlled 55 C**	32.9	6.6	2.8	6.9	38.1
versus					
900 m^3/h/Mg constant aeration	29.0	0.7†	4.6	3.1	37.7
900 m^3/h/Mg constant aeration	29.4	6.3	4.1	2.8	41.3
versus					
1800 m^3/h/Mg constant aeration	29.4	6.3	3.9	3.1	39.8
900 m^3/h/Mg constant aeration	22.1	5.3	10.6	2.4	49.3
versus					
Temp. demand aeration	45.3	12.2	21.7	5.9	33.0

* g CO_2-C lost per 100 g dry initial mixture
** 20 cm diameter cylinder
† error due to inaccurate moisture determination

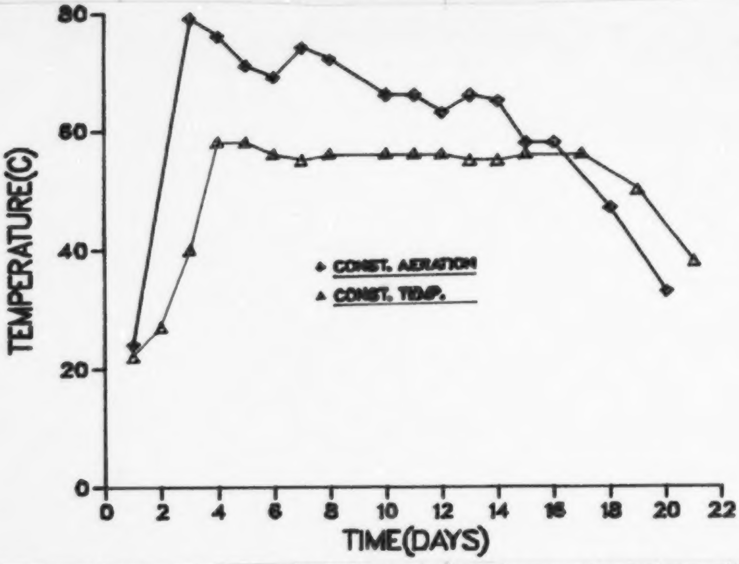

Fig 22. Temperatures recorded from laboratory composter studies in which both had aeration rates of 900 m^3/h/Mg and one had a 55°C temperature limit on the insulating bath.

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coincidental, the question remains as to why both composters cooled simultaneously. Because moisture was not limiting in either case, a depleted energy source was the probable cause of the temperature decline. Carbon dioxide data indicated that the 55°C composter produced 50% more CO_2 than the normal composter probably by having a more diverse population to degrade a greater variety of compounds in the mixture (Fig. 23). A more diverse population is characteristic of the cooler temperature composting systems (Golueke et al., 1954).

High Constant Aeration Rate Study

To control the temperature during composting with aeration, using the laboratory system, it was necessary to build a smaller, inner mesh cylinder. A cylinder with one-half the diameter and one quarter the volume of the 20 cm cylinder was used in subsequent studies. The first studies in which the smaller cylinders were used involved testing the aeration rates the 900 and 1800 $m^3/h/Mg$, dry weight (all aeration rates reported are based on dry weight) in keeping temperatures from exceeding 60°C. The higher aeration rate kept the temperature from exceeding 60°C but, also, led to rapid cooling (Fig. 24). The lower aeration rate resulted in maximum temperature of approximately 65°C, a rapid cooling of the material, but a slightly longer composting run than the higher aeration rate. Note that secondary temperature peaks were not present in either composter although the CO_2 data indicated that a secondary CO_2 peak was starting to appear at the end of the 900 m^3/h Mg study (Fig. 25 and 26). The total amount of CO_2-C lost from both systems was nearly

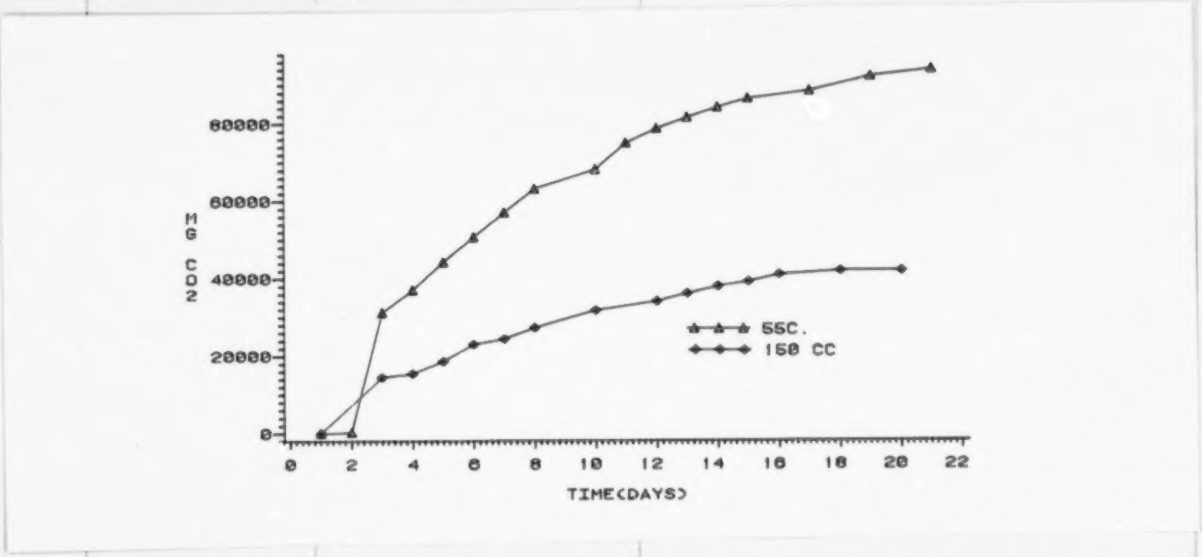

Fig. 23. Cumulative CO_2-C from laboratory composter studies in which both had aeration rates of 900 $m^3/h/Mg$ and one had a 55°C temperature limit on the insulating water bath.

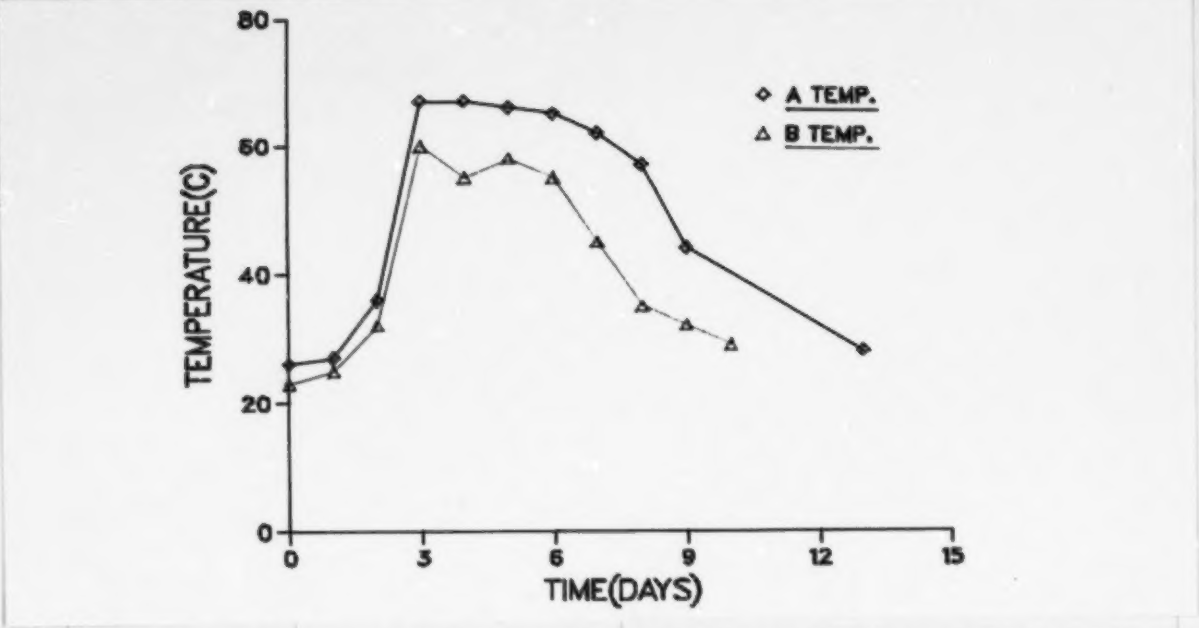

Fig. 24. Temperatures from laboratory composters with 10 cm cylinder and aerated at 900 $m^3/h/Mg$ (A) or 1800 $m^3/h/Mg$ (B).

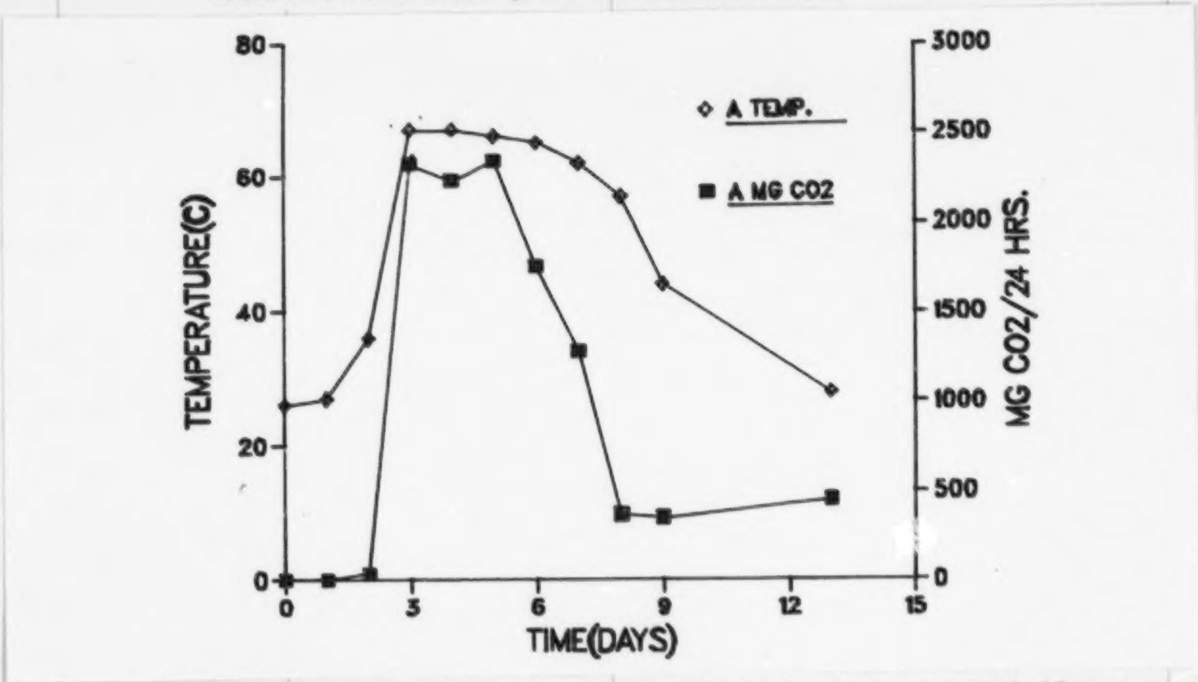

Fig. 25. Temperature and CO_2-C data from laboratory composter with 10 cm cylinder and aerated at 900 $m^3/h/Mg$.

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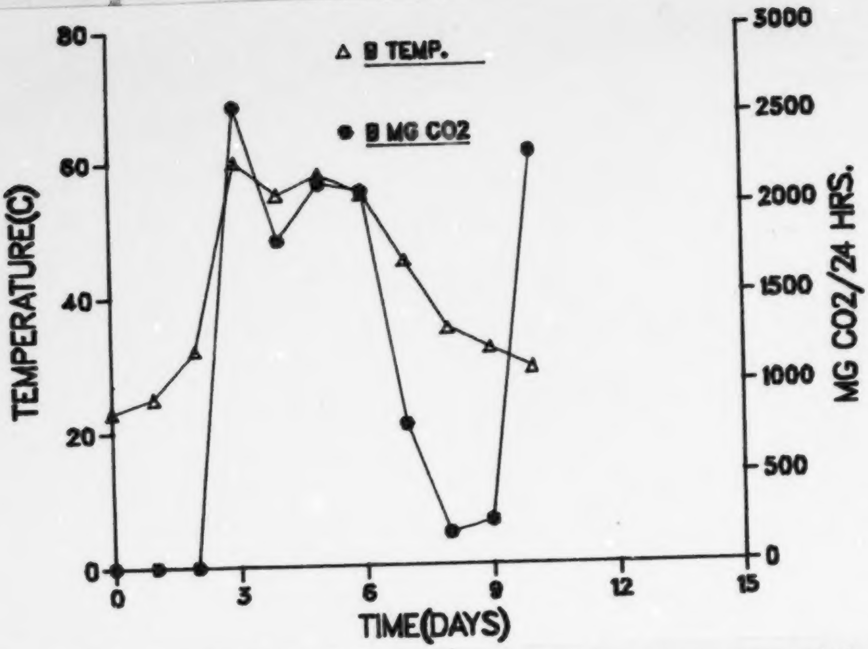

Fig. 26. Temperature and CO_2-C data from laboratory composter with 10 cm cylinder and aerated at 1800 $m^3/h/Mg$.

equal indicating that the flow rates used in this study were sufficient to overcome any diffusion limitations that may be suggested from using this type of aeration system (Fig. 27).

The aeration rate study provided important information on the loss of NH_3 in relation to CO_2 during composting. The initial studies performed using the self-heating composter indicated that NH_3 evolution lagged behind CO_2 by a few days (Fig. 21). In the study using 900 m^3/h Mg aeration rate, a similar trend was observed but with only a one day lag period (Fig. 28). However, at the 1800 $m^3/h/Mg$ aeration rate the lag period was not present (Fig. 29) and the cumulative condensate was considerably greater (Fig. 30). These data indicated that as the condensate removal became more efficient, the CO_2 and NH_3 evolution curves appeared more to be simultaneous that in sequence.

The data obtained from the aeration study indicated that continuous rapid aeration did reduce peak temperatures, but also reduced the duration of the composting process. When comparing the cumulative CO_2-C loss from these systems to the loss from the composter study where temperatures were held at 55°C, significantly less CO_2 evolved per 100 g of mixture from the 900 and 1800 $m^3/h/Mg$ studies even though the sludge was from the same source (Table 12). These data, indicated the possibility that continuous, rapid aeration cools the compost too rapidly so that the microbial population whose activity normally appears as a secondary temperature peak during the cooling phase does not have time to develop

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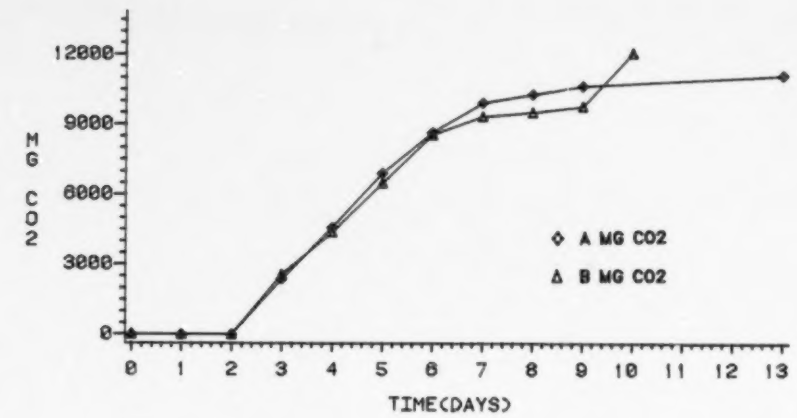

Fig. 27. Cumulative CO_2-C from laboratory composter with 10 cm cylinder and aerated at 900 $m^3/h/Mg$ (A) or 1800 $m^3/h/Mg$ (B).

TABLE 12. MATERIALS LOSSES FROM RAW, LIMED SEWAGE SLUDGE AND WOODCHIP MIXTURE COMPOSTED IN LABORATORY COMPOSTER.

Analyses performed	Loss %
Total Wet Weight	35.4
Total Dry Weight	10.1
Volatile Solids	14.7
Total Kjeldahl N	12.5
Total CO_2-C *	54.7

* grams

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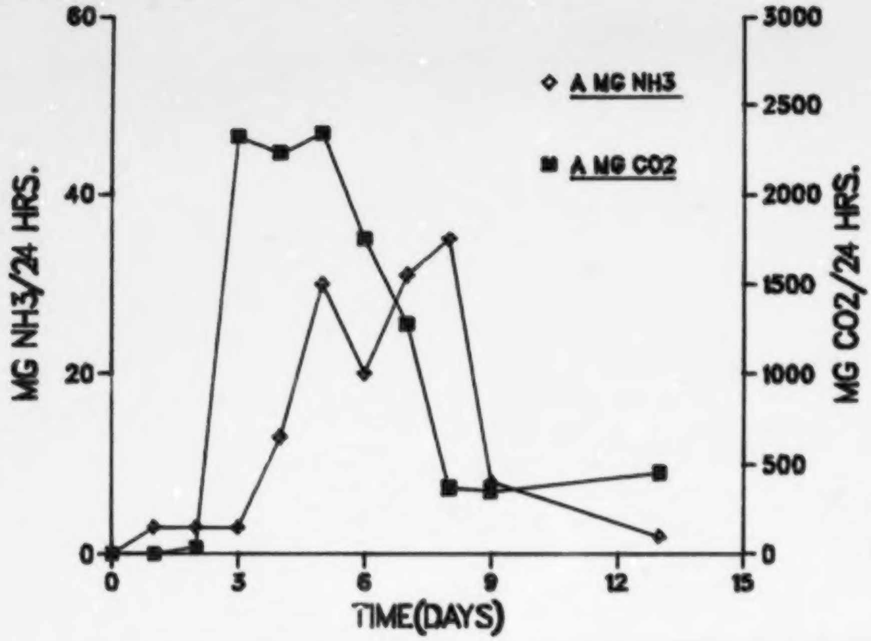

Fig. 28. Carbon dioxide-C and NH_3-N loss with time from laboratory composter with 10 cm cylinder and aerated at 900 m^3/h/Mg.

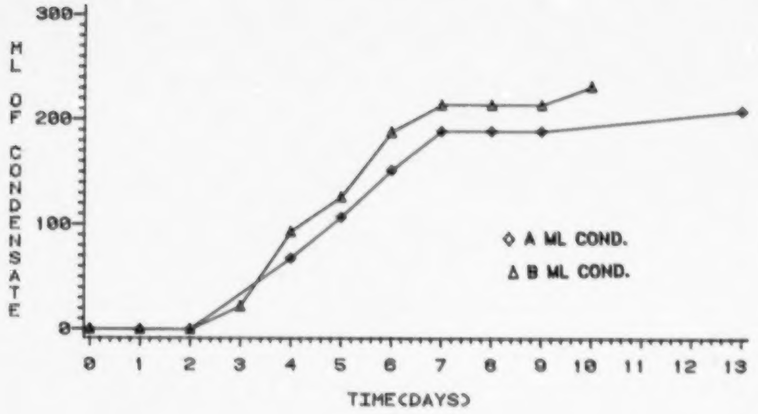

Fig. 29. Cumulative condensate from laboratory composters with 10 cm cylinder and aerated at 900 m^3/h/Mg (A) or 1800 m^3/h/Mg (B).

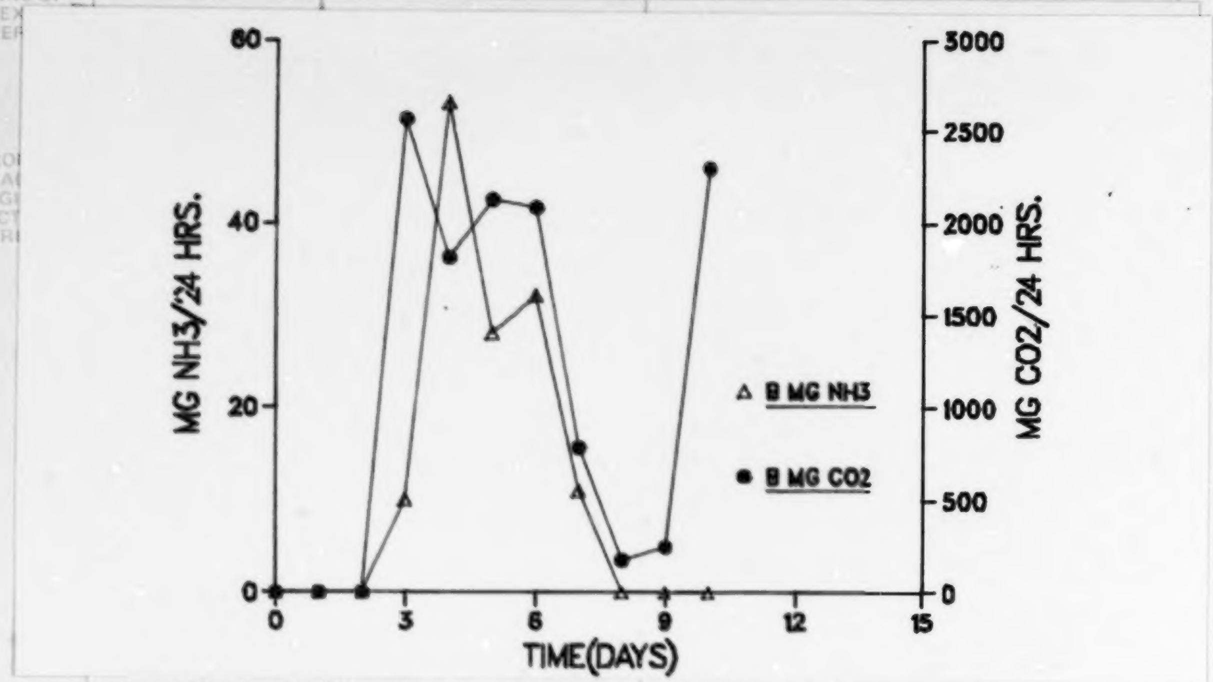

Fig. 30. Carbon dioxide-C and NH_3-N loss with time from laboratory composter with 10 cm cylinder and aerated at 1800 m^3/h/Mg.

(Wiley and Pearce, 1957). It is our estimation that the secondary peak in our studies is probably the result of a microbial population appearance or reappearance during the composting process. Using the self-heating composter several studies involving various substrates resulted in the appearance of secondary peaks including a beef cattle manure-straw mixture. Considering the variations in substrates but still the presence of a secondary peak during composting indicates that temperature is the key variable and not substrate which controls the appearance of a secondary peak during the cooling phase of the composting process. It also appears that if the cooling is too rapid, the population does not have sufficient time to develop and a secondary temperature peak will be absent.

High Constant Aeration Versus Temperature Demand Aeration

A third composter study was initiated to directly compare composting of sludge under a constant aeration of 900 m^3/h/Mg with that of a temperature demand system. Under the latter, aeration was manually controlled to keep temperatures near 55°C. Aeration rates at the start of the experiment were identical for both composters.

The data indicated that temperatures resulting from constant aeration which was the control in this study were similar to those previously discussed. After a short delay, temperatures rose to 70°C and declined quickly with no secondary temperature peak (Fig. 31). The run was completed in 15 days. The temperature demand curve showed that the

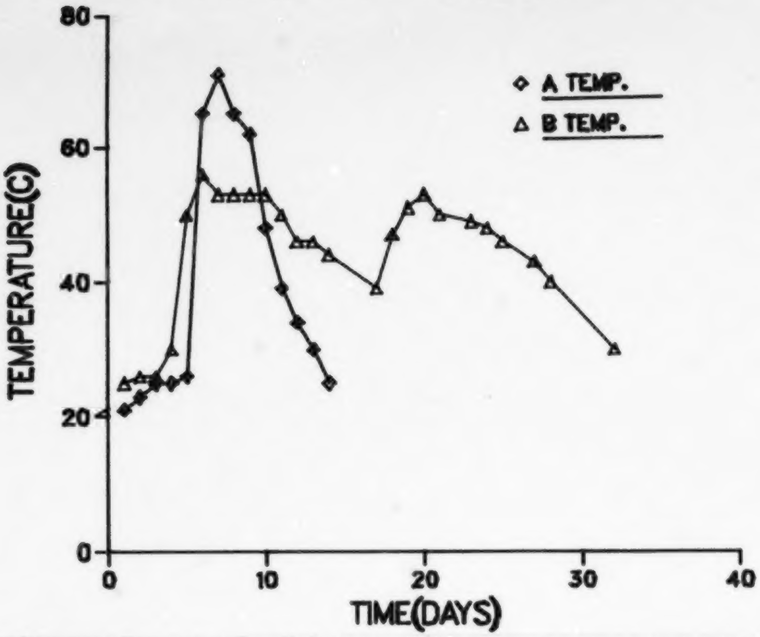

Fig. 31. Temperatures recorded from laboratory composter with 10 cm cylinder and aerated at a constant rate (900 $m^3/h/Mg$, A) or by temperature demand (B).

aeration system was successful in keeping temperatures below 60°C. Aeration was increased in response to temperature increases starting on day 4 from 900 to 3600 $m^3/h/Mg$ on day 6 and decreased slowly to 900 $m^3/h/Mg$ on day 10. Aeration remained constant till day 19 when it was increased to 1200 $m^3/h/Mg$ and decreased to 600 to 900 $m^3/h/Mg$ on day 21 where it remained for the duration of the study. The temperature demand study lasted nearly 3 weeks longer than the constant aeration study which indicated that substantial amounts of decomposeable substrate still remained in the constant aeration composter after it had cooled to ambient temperature. The evolution of CO_2 corresponded to the increases and decreases in temperature (Fig. 32 and 33). At peak activity the temperature demand system produced twice as much CO_2 as constant aeration system. Cumulative CO_2 levels indicated that the temperature demand composter decomposed 2.5 times more substrate than the constant aeration composter (Fig. 34). On a 100 g basis, the amount of CO_2-C evolved from the temperature demand study was slightly less than from the composting study discussed previously when the temperature limit of 55°C was imposed on the water bath (Table 12). The final moisture content of the compost from the temperature dependent study was 33% which might be near the limit for detectable microbial activity.

The loss of NH_3 from the constant aeration composter lagged one day behind CO_2 evolution (Fig. 35). In the temperature demand study, however, peak aeration, peak decomposition, NH_3-N loss occurred simultaneously (Fig. 36). These data support the previous conclusion that

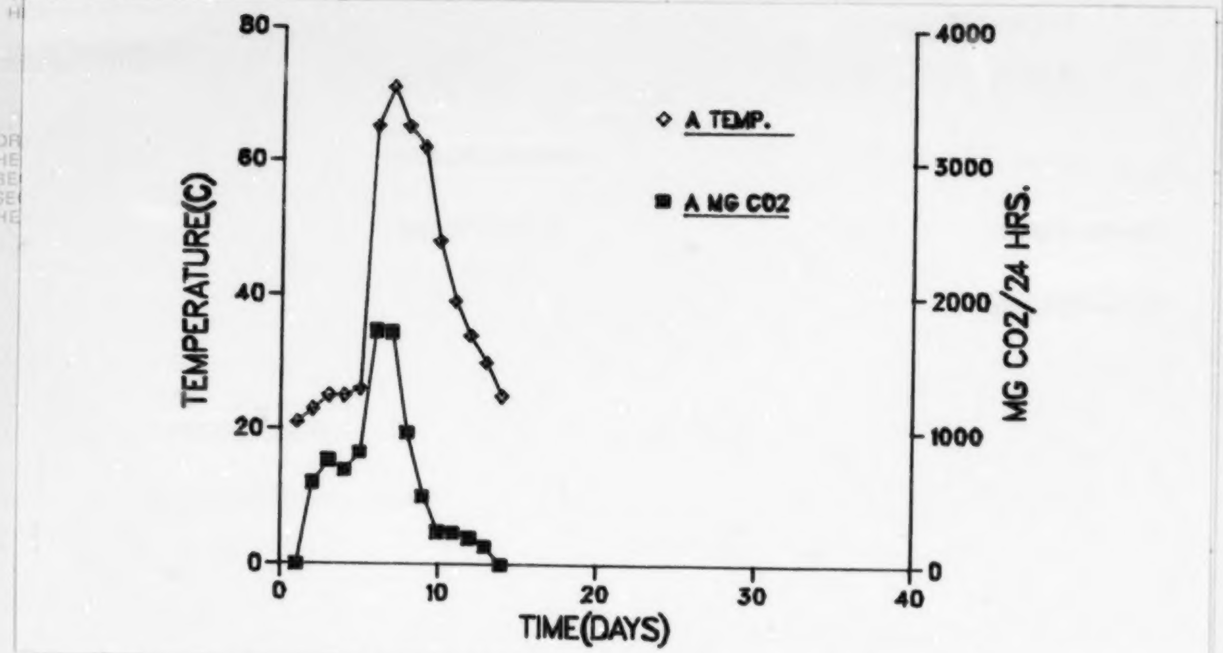

Fig. 32. Temperatures and CO_2-C evolution with time from laboratory composter with 10 cm cylinder and constant aeration.

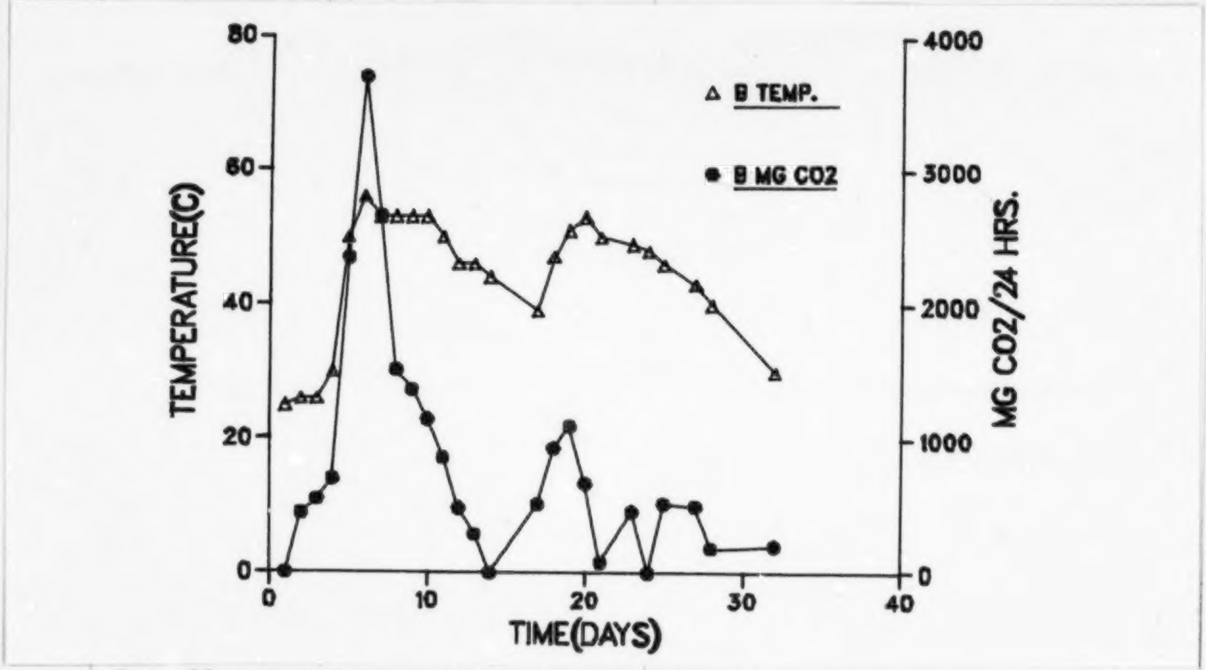

Fig. 33. Temperatures and CO_2-C evolution with time from laboratory composter with 10 cm cylinder and temperature demand aeration.

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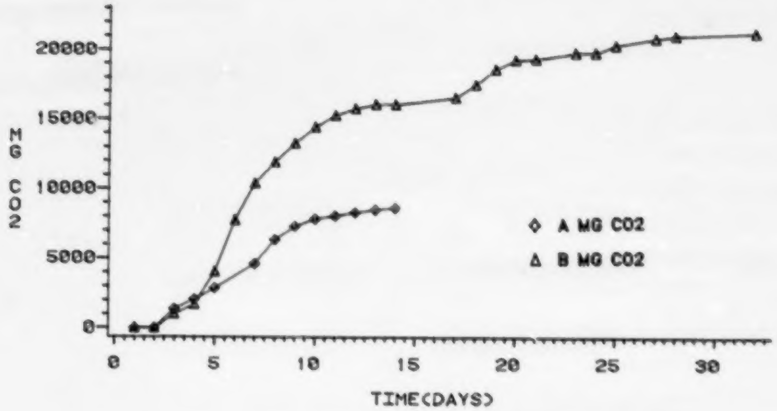

Fig. 34. Cumulative CO_2-C from constant aeration (A) and temperature demand aeration (B) composters.

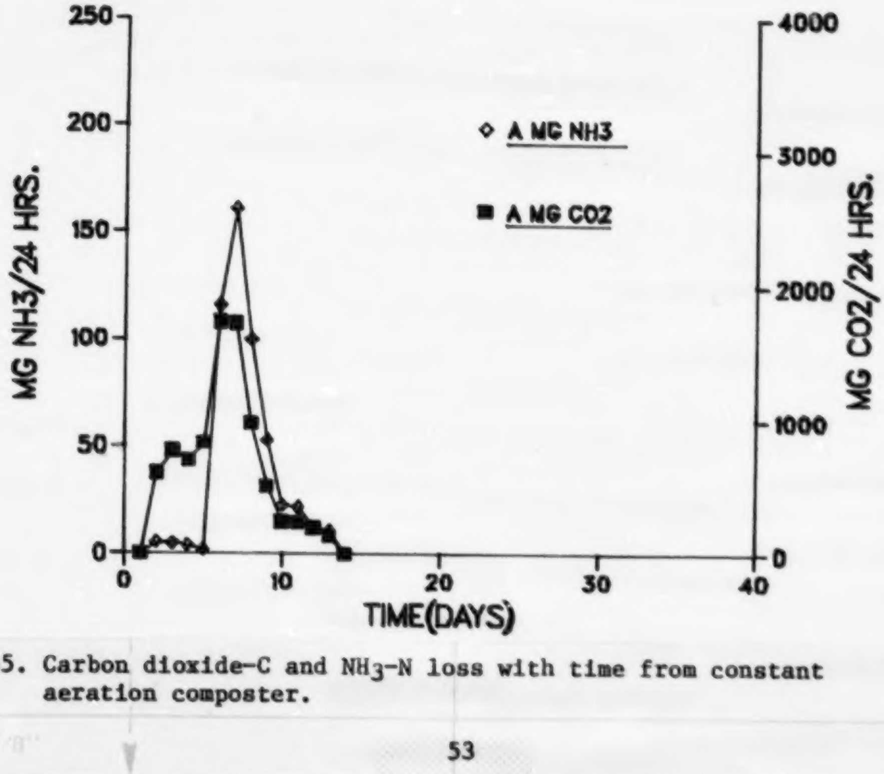

Fig. 35. Carbon dioxide-C and NH_3-N loss with time from constant aeration composter.

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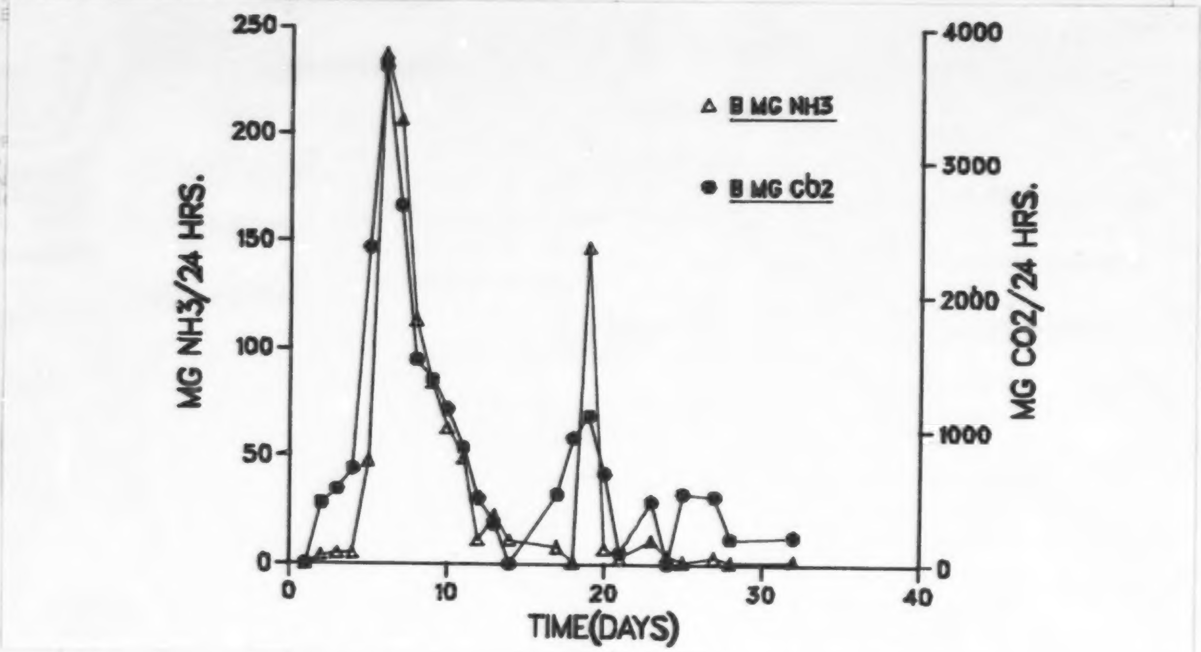

Fig. 36. Carbon dioxide-C and NH_3-N loss with time from temperature demand aeration composter.

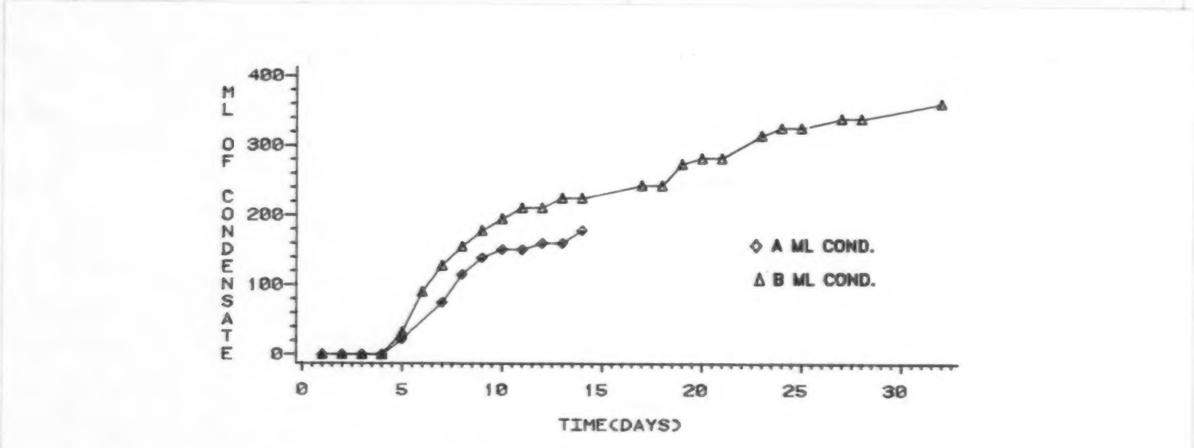

Fig. 37. Cumulative condensate from constant aeration composter (A) and temperature demand aeration composters (B).

the reason for NH_3 loss lagging 1 to 4 days behind CO_2 evolution in previous studies was insufficient aeration to remove the NH_3-containing condensate.

The cumulative condensate losses from the constant and temperature demand systems were not as dramatically different as were the CO_2 levels (Fig. 34 and 37). At day 14, the time when the constant aeration study was completed, the difference in moisture loss was only 20% more for the temperature demand composter. The reason for this occurence, even though the flow rates were one fourth that in the temperature demand study, was that the temperature of the constant aeration composter was significantly higher. The relationship between water removal and volume of air at a constant temperature is linear while the relationship between water removal and air temperature is exponential (Haug, 1980).

Microbiological analyses of the sludge-woodchip mixture before and after composting was performed as part of this experiment to determine changes in the type and number of microorganisms during composting. The analysis of mixtures before and after composting that had been subjected to temperature demand aeration indicated a dramatic increase in biomass (Table 13). Bacteria, fungi, and actinomycete populations rose 3 to 5 orders of magnitude over that in the initial mixture. The compost mixture in the temperature demand cylinder was grey with a thick microbial mat over sludge-woodchip mixture. The bacterial and actinomycete population increased in the constant aeration mixture. The notable difference between the constant aeration and temperature demand compost mixtures was the fungal population. Temperatures near 70°C which were recorded in the constant aeration system reduced fungal population to below detectable limits. The bacterial and actinomycete population increased more in the temperature demand mixture indicating that the temperatures were also more favorable for these microorganisms. The microbial data indicated that high temperatures (>65°C.) decreased both total number and diversity of population which resulted in a substantial decline in CO_2-C evolution or stabilization.

DISCUSSION

The self-heating laboratory composter provided insight into the relationship between aeration, water loss and temperature during composting. When the sludge-woodchip mixture was allowed to self-heat and cool under an aeration rate similar to that used in field-scale composting, temperatures were similar to those recorded in the field. Peak temperatures were near 80°C and as temperature declined, a secondary temperature peak occurred. Several investigators have noted the occurrence of secondary peaks (Ashbalt and Line, 1982; Milner and Geddes, 1946; Chang and Hudson, 1967; Sikora et al, 1983). The consensus of opinion as to the cause for the secondary temperature peak is a recovered themophilic microorganism population. Because secondary temperature peaks have been noted in composting of several varied substrates (wheat straw, manure, sewage sludge, grain), the hypothesis that a specific class of substrate becomes available for degradation at the time the secondary peak occurs appears unlikely. Also, as noted in the aeration study, rapid aeration

TABLE 13. MICROBIAL COUNTS IN RAW LIMED, SEWAGE SLUDGE-WOODCHIP MIXTURE BEFORE AND AFTER COMPOSTING UNDER CONSTANT OR TEMPERATURE RESPONSE AERATION.

Sample	Bacteria	Fungi	Actinomycetes
	---- Microorganisms . g^{-1} compost ------		
Initial Mixture	$3.6 \pm .48 \times 10^6$	$1.6 \pm .27 \times 10^4$	$9.5 \pm 5.2 \times 10^5$
After Constant Aeration	$3.6 \pm .5 \times 10^8$	<200	$4.2 \pm 3.0 \times 10^7$
After Temperature Response Aeration	$3.3 \pm .34 \times 10^9$	$3.6 \pm .97 \times 10^8$	$3.3 \pm 1.8 \times 10^9$

would lead to a relatively rapid decline in temperature and a secondary temperature peak was not found. The rapid decline in temperature should not have affected the availability of a substrate as it would the appearance of a microbial population with a narrow optimum temperature range. Therefore, the appearance or reappearance of a population with a specific thermophilic temperature range appears to be the reason for the secondary CO_2 peaks in compost piles.

In the study where the temperature of the mass was held to below 60°C by placing a limit on the water bath, more than twice the CO_2 was produced than from the companion study where the mass was allowed to self-heat and cool (Table 11). Yet the length of time for both systems was nearly equal. The aeration rate was not sufficient to reduce the moisture to limiting levels in this study and, therefore, the reason for both composters to cool was an exhausted energy source.

Constant, high aeration did reduce peak temperatures but did not result in the most efficient composting system. Constant, high aeration removed water and cooled the mass rapidly. The most efficient system was the temperature demand aeration system. Increasing and decreasing the aeration rates to keep temperatures between 50 and 55°C, allowed the maximum thermophilic population to remain active for extended periods of time.

The microbial analyses performed on the samples showed results similar to other studies (Gray et al., 1971a, Finstein and Morris 1975). The disappearance of fungi from the high temperature composts and the significant difference in CO_2 evolution underlines the importance of fungi in the stabilization process and the necessity of a successful compost operation to control temperatures as much as possible.

The amount of condensate or moisture removed is dependent upon volume of air and temperature of the mass. Doubling the aeration rate from 900 to 1800 $m^3/h/Mg$ did not double the condensate loss from the system. In fact only slightly more condensate was removed at the high aeration rate indicating that lower aeration rate was suitable to remove all the condensate available (Fig. 30). Decomposition is linearly related to moisture removal only under conditions of equal temperature and sufficient volumes of air to remove the condensate. The temperature demand study, as an example, indicated that after fourteen days, only 20% more condensate was removed from the temperature demand cylinder receiving several liters more air than the constant aeration system even though over twice as much CO_2 evolved from the temperature demand cyclinder. The reason for this result is the exponential relationship between water removal and temperature. Therefore, the concept of stabilization equalling moisture removal or drying in a composting system must be considered with equal temperatures in mind.

Nitrogen loss from organic material composting has not been studied extensively and from the data presented here, amount of N lost is proportional to condensate loss. In the temperature demand study, nearly 20% of the total N was lost during composting which was significantly more than observed in previous studies (Sikora et al, 1983). A considerable amount of organic matter was also decomposed in this study which resulted in a final product with nearly the same total N concentration as the starting material (1.32 versus 1.35%). Therefore, increased aeration leads to increased loss of nitrogen but, with the increased loss of volatile solids, the N content of the final product changes only slightly.

SECTION 8

EFFECT OF NUTRITIONAL AND NON-NUTRITIONAL FACTORS ON THE GROWTH OF ASPERGILLIS FUMIGATUS (AF) AND NATURAL SOURCES OF AIRBORNE AF AND THERMOPHILIC ACTINOMYCETES

INTRODUCTION

The potential public health risk associated with the aerosolization and inhalation of spores of *Aspergillus fumigatus* at sludge-composting sites has been a concern to those who are responsible for the planning and operation of compost facilities (Millner et al., 1977; Marsh et al., 1979; Millner et al., 1980; Passman, 1980). The presence of the fungus in air at compost sites is not in itself unusual because the organism is adapted to growth on organic substrates subjected to self-heating (Cooney and Emerson, 1964; Emmons, 1962; Tansey, 1971; Kane and Mullins, 1973; Lacey and Lacey, 1964). However, by their dispersal in air from the compost site to adjacent areas, these spores can constitute a source of infectious agent to susceptible individuals. Hospitals and other health-care facilities in the area around compost sites would contain a concentrated population of susceptible individuals. In such cases, some evaluation of the impact that airborne spores of *A. fumigatus* (AF) would have on the public health should be made. This would conceivably require knowledge and information about 1) the expected concentrations of airborne AF at locations adjacent to the emitting sources, 2) the susceptibility of the host populations, and 3) the dose-response relationships.

Determinations of the first factor can be estimated using atmospheric dispersion models (Turner, 1964) and data from existing compost sites (Millner et al., 1980; Passman, 1980; Clark et al., 1983; Jones and Cookson, 1983). Information about susceptibility of individuals is available in several reviews (Marsh et al., 1979; Joseph, 1983). However, data and information about dose-response relationships are lacking. Information about the ambient levels of AF spores in air could be used to assist in the evaluation of the potential impact of the aerosols on public health in adjacent areas.

The apparent ubiquity of AF has been noted, but the relative differences in amounts of AF at different ambient sites is more difficult to evaluate from literature records. Presumably, susceptible individuals come into contact with AF during the normal course of their daily activities. However, the level of AF in different environments could be expected to vary depending on the availability of substrate and on temperature conditions.

The work reported here was undertaken in order to increase the database concerning the quantitative differences in airborne levels of AF under environmental circumstances that affect the growth of the fungus. To this end, an extensive survey of literature records was made to determine what was already known about the occurrence of AF in air. Volumetric data are limited (Table 14). Then, a series of air-samples collected from various locations, including some suspected natural sources, were analyzed for viable AF.

TABLE 14. RECORDS OF VOLUMETRIC CONCENTRATIONS OF A. FUMIGATUS (AF) IN AIR

Concentration (Af/m^3)	Location*	Sampling Apparatus	Reference
0.25 - 7.0	London, (Britain (o)	Hirst	Austwick, 1963
0.3-.2300	hospital, Britain (i)	slit sampler	Noble & Clayton,1963
0.115-0.219	orchard, Britain (o)	Andersen	Hudson, 1969
4000	silo, Britain (o)	Andersen	Lacey, 1971
120	Rothamsted, Britain(o)	Rotorod	Lacey, 1975
6-34	urban, Britain (o)	Andersen	Mullins, 1976
0-210	1 m from static compost pile, Britain (o)	Andersen	Mullins, 1976
0-45	31 m from static compost pile, Britain	Andersen	Mullins, 1976
1.7	Kansas, USA(i)	Pady-Rittis slit	Kramer et al., 1960
0-150	Michigan, USA (i&o)	Andersen	Solomon & Burge, 1975
0-123	hospital wards (i)	Andersen	Solomon, et al., 1978
(median 1.2)	Michigan, USA		
0-67	hospital	Andersen	Solomon et al., 1978
(median 3.5)	Michigan, USA (o)		
384	chick hatchery,Iowa, USA(i)		Chute & Barden, 1964

*i=indoors; o=outdoors.

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In addition to the above, studies were made of the nutritional and non-nutritional factors, relevant to the composting of sewage sludge, that affect the growth of AF. The objective of this part of the work was to determine whether or not there was a basis for trying to manage the composting process so that there would be a minimal growth and aerosolization of AF.

Finally, in connection with the concern about exposure of compost-site workers to farmer's lung antigens (thermophilic actinomycetes), determinations of the levels of these microbes in air were made concurrently with those that were made for AF in the different ambient environments. Previous to the studies reported here, thermophilic actinomycetes had been repeatedly isolated from air samples collected at two sewage-sludge composting sites. However, the numbers and kinds had not been adequately assessed. Inhalation of these microbes is known to cause a progressive and disabling deterioration in the pulmonary function of sensitive individuals. The disorder has been termed hypersensitivity pneumonitis, allergic alveolitis, farmer's lung, and several other names (Burge and Millner, 1980). As with AF, the thermophilic actinomycetes proliferate in conditions, of high temperature and adequate substrate and moisture (Lacey et al. 1972).

Review of the Ecology and Physiology of *Aspergillus fumigatus*

The fungus *Aspergillus fumigatus* is important in the deterioration of organic materials and as an opportunistic pathogen of man. (Emmons et al., 1970; Rippon, 1974). Information about its biology and natural occurrence, including its general distribution, physiological attributes, cultural characteristics and sensitivity to biocides, provides a perspective in which the results reported here can be placed. Available information about the fungus is summarized below.

Ecology and General Distribution--

Aspergillus fumigatus is a member of a large fungus genus which includes approximately 300 species, many of which have been isolated from a wide variety of substrata and localities (Raper and Fennell, 1965; Rippon, 1974); *Aspergillus* has been referred to as a genus with a cosmopolitan distribution. The pervasive occurrence of aspergilli in the environment has provided support for the use of the cliche "our moldy earth." However, reference to the ubiquity of aspergilli requires recognition of the fact that not all species occur in all places at the same frequency and concentration. Biotic and abiotic factors affect the absence, presence, and abundance of species, such as *A fumigatus*, relative to the abundance of the genus. Gregory (1973) in his treatise on the microbiology of the atmosphere, comments on these and another consideration with regard to airborne microbes (p. 478): "The well-known microbiological doctrine that all kinds of microbes are universally distributed and are merely selected by the substrate is not universally true, and when applied to pathogens is dangerous."

The habitat from which AF has been most consistently isolated is organic material undergoing aerobic biological decomposition at high temperature (Cooney and Emerson, 1964; Emmons, 1962; Tansey, 1971). Its isolation from soil and air relates to its ability to grow on a variety of substrates located in suitable conditions. Some examples of substrates on which *A. fumigatus*, along with many other types of fungi, have been found to grow and cause deterioration follow.

Wood--AF causes 'soft-rot' which results from the degradation of the cellulose in the dead cells. Only slight modification of the lignin component of wood occurs with soft-rot. Birch, pine, and spruce sapwood incubated at 40°C with AF showed loss in weight of 3.8%, 3.5%, and 2.9% respectively (Kaarick, 1974). AF has also been isolated from fungicide-treated beech-wood stakes (Sharp, 1975)

Paper--As a wood decay fungus, AF grows in wood pulp piles awaiting paper processing. The organism has also been implicated in the 'slime' formation problem in paper processing waters (Hughes, 1968).

Cotton--Turner (1967) found AF growing on and damaging raw cotton fiber. Species of the *A. fumigatus* group are cellulose degraders (Reese and Downing, 1951). Production of fungal protein from cellulose and cellulosic wastes has been accomplished using pure cultures of AF (Rogers et al., 1972).

Leather--In the tropics, AF has been found growing on vegetable tanned leather products, which contain high levels of sucrose (Turner, 1967; Orlita, 1968).

Rubber and Plastics--Cundell and Mulcock (1972) found AF growing on rubber, but also found that it was unable to degrade pure rubber hydrocarbon. The utilization of plasticizers of natural origin, e.g. castor oil is a major cause of biodeterioration in plastics. Especially used in the vinyl industry, plasticizers alter the secondary forces between polymer chains and increase the flexibility of the plastic (Thomas, 1977). Degradation or bio- utilization of the plasticizer causes increase in brittleness of the plastic (Klausmeier and Jones, 1961). Thermophilous fungi, including *A. fumigatus*, can attack plasticizers, even those which are resistant to attack by mesophilic fungi (Mills and Eggins, 1974). The hydrophilic polymer of contact lenses is subject to attack by AF (Filippi et al., 1973).

Aviation fuel--*Aspergillus fumigatus* was found in fuel tanks of aircraft that heat to 40 to 45°C. This condition frequently occurs in fuel tanks of supersonic transport aircraft (Thomas, 1977).

Glass--Under humid conditions, glass in optical lenses of instruments has been etched by the organic acids produced by fungi growing on the oils, mites and other insects, and other organic debris found on the surfaces. *Aspergillus fumigatus* is one of several *Aspergilli* which have been found growing in this condition (Turner, 1967).

Atmosphere--Although fungi do not grow in air alone, i.e. conduct an entire life cycle, their presence or more precisely the presence of fungal spores in air has been a subject of considerable interest to plant pathologists as well as allergists. Efforts have been made from time to time to determine distribution and frequency of fungal spores in the air.

Many airspora studies report the occurrence of colonies identified as *Aspergillus* spp. on gravity settle plates exposed to air. Mullins (1974), in an analysis of 94 airspora surveys, noted that "the genus *Aspergillus* was recorded between first and fifteenth in order of incidence of airborne moulds and varied in frequency from 0.1 to 22% of the total air spora samples" (Mullins et al., 1976). Our survey of this airspora literature (Table 1) and our test results in general support these summary statements made by Mullins (1974).

Despite the clinical significance of AF, i.e. its ability to grow in various parts of the body under certain circumstances, very few airspora studies, either volumetric or gravity settle plate, have reported the presence of AF specifically.

The limited number of reports of volumetric data for AF which are available come in part from Britain and in part from the U.S. These are shown in Table 14.

In general, the records show that AF can be isolated from substrata or air in almost every location, but predominant occurrence or high concentration are likely to be found in only localized circumstances, especially those in which high temperature (40-60°C) prevails. Our test results agree with this general conclusion.

Physiological Attributes and Cultural Characteristics--

Strains of AF from patients are similar morphologically and physiologically to wild type strains isolated from nonhuman sources (deVries and Cormane, 1969). Their results on 119 test strains of *A. fumigatus* show that glucose, fructose, saccharose, and maltose were used by all strains. Dextrin, galactose, and glyceros were utilized by only some strains; lactose and potassium pyruvate were not well utilized. The nitrogen sources, KNO_3, NH_4^+, NO_3^-, urea, glycine, l-alanine, l-glutamine, tryosine, and peptone were all good suitable for growth, but cystine was not. All strains liquified gelatin. Growth was unaffected by the presence or absence of 9 supplemental vitamins. Ten of 30 isolates tested grew well at 37°C in the absence of O_2. Seven of eight isolates tested in 5% CO_2 atmosphere at 37°C produced an effuse mycelial growth but no sporulation. Inhibitory effects occurred with amphotericin B, pimaricin, nystatin, trichomycin, but not with griseofulvin.

The limiting effects of moisture on fungus growth and spore germination were discussed in detail by Griffin (1963). With regard to AF, he cited Galloway's work which showed that AF spores germinated after 2 weeks when incubated at 25°C with 85% relative humidity. Ayerst (1969) showed that 40°C is the optimum temperature and 97% is the optimum relative humidity for growth of AF. The limiting temperatures for growth of the 2 AF strains he studied were 12°C and 53°C; relative humidity of 82% was the least which would permit growth of AF.

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The survival of spores at high temperatures has been investigated by Busschert et al., (1968). His results show that a 90% reduction in viable AF spores follows exposure at 50°C for 11,596 min., at 55°C for 440 min., and at 60°C for 16.8 min.

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In a study of the physiology of spore germination for AF, Campbell (1971) showed that spore swelling, an essential state in the germination process, required an external source of assimilable carbon. Further, the concentration of the external carbon source exerts a greater influence on germination than external nitrogen sources. Germ tube emergence, a certain sign of success of the germination process, was observed to occur usually between 6-9 hours after the start of incubation at 40°C in glucose-peptone broth.

Biocide Sensitivity--Studies by Russell et al. (1975) revealed that ungerminated spores were insensitive to the antibiotic amphotericin B methyl ester. However, sensitivity developed during spore swelling and was maintained throughout the emergence of the germ tube.

In a 12-month study of the effects of fungicides on the occurrence of thermophilous leaf-surface fungi (Kuthubutheen and Pugh, 1977) AF was consistently isolated. With monthly treatments of grass (*Agrostic tennis*) with the fungicides Captan (9 kg/ha), Dicloran (2 kg/ha), Thiram (16.7 kg/ha), AF was isolated in 87.5%, 93.8%, and 81.25% of the samplings; with Verdasan (12.5 kg/ha), AF was isolated in only 75% of the samplings.

EXPERIMENTAL PROCEDURES AND MATERIALS FOR FIELD OPERATIONS

Air Sampling for Fungi and Thermophilic Actinomycetes

Habitats in which environmental conditions conducive for the growth of *Aspergillus fumigatus* (AF) and thermophilic actinomycetes (TA) were selected for study. Conditions required were high amount of available moisture, potential for temperatures above 35°C, suitable substrates, especially cellulosic and complex organic matter. The major objective of this study was to determine whether the microbial content of the air around such sites reflects the natural source of AF and TA and is thus indicative of sites of localized self-heating. The potential for seasonal fluctuations in the microbes at the sites was considered.

Air samples were obtained by using Andersen six-stage, viable (microbial) particle samplers (Andersen 2000, Inc., Atlanta, GA). The samplers operate volumetrically and fractionate particles by impaction into six groups based on the equivalent aerodynamic mass of the particles. The aerodynamic sizes of the collected particles correspond to the stages of the sampler in the following way: stage 1, 7.0 um; stage 2, 4.7 - 7.0 um; stage 3, 3.3 - 4.7 um; stage 4, 2.1 - 3.3 um; stage 5, 1.1 - 2.1 um; stage 6, 0.65 - 1.1 um. In the human airways, certain areas of deposition correspond to the different size fractions: nasopharynx, 4.7; trachea and primary bronchi, 3.3 - 4.7 um; secondary bronchi, 2.1 - 3.3 um; terminal bronchi, 1.1 - 2.1 um; alveoli, 0.65 - 1.1 um (Anderson, 1958).

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Concentrations of AF and TA in air were determined for each of the sampling sites listed in Table 15. Sites listed represent 21 outdoor and indoor suspected sources and three nonsuspect (reference) sources. The annual sampling schedule was divided into quarters corresponding to the seasons: Fall -- September 21 to December 20; Winter -- December 21 to March 20; Spring -- March 21 to June 20; Summer - June 21 to September 20. Samples at the 3 reference (nonsuspect) sites were taken once per week for 9 weeks during each quarter. Three samples at other outdoor and indoor sites, starting with #1 in Table 2 were taken each week.

Each location was sampled separately for AF and TA. The nutrient medium for AF collections was oxgall-gentamicin prepared as previously described and with incubation at 45°C (Millner et al., 1977). For TA, trypticase soy agar with 50 mg/L cycloheximide was used with incubation at 50°C. (All agars and media are Difco unless otherwise specified).

For outdoor sites, the sampler was placed on its side in a retainer positioned at approximately 2.0 m above ground level with the sampler oriface oriented in the mean wind direction. With the cone-shaped cover removed, as

TABLE 15. LOCATIONS FOR AIR SAMPLING (OUTDOORS/INDOORS)

1. Barnyards - USDA, Beltsville, MD - dairy
2. Barn - USDA, Beltsville, MD - dairy
3. Cornfield - USDA, Beltsville, MD
4. Grassy lawn - Columbia, MD
5. Mulched Landscape Planting I (residence) - Columbia, MD
6. Mulched Landscape Planting II (hospital) - Takoma Park, MD
7. Poultry house - USDA, Beltsville, MD - turkey house
8. Garbage bin - Beltsville, MD
9. Park with mulch - Brookside Botanical Gardens, Wheaton, MD
10. Mixed hardwood forest - Wheaton, MD
11. Dusty book library - National Agricultural Library, Beltsville, MD
12. Greenhouse - USDA, Beltsville, MD
13. Plant nursery - Beltsville, MD
14. Boiler room - USDA, Beltsville, MD
15. Zoo - bird cage - National Zoo, Washington, D.C.
16. City park - Washington, D.C. - Mall
17. Attic - Silver Spring, MD
18. Basement - Silver Spring, MD
19. Refuse/garbage dump - Bowie, MD
20. Mushroom house - Kennett Square, PA
21. Coal waste piles - self-heating - Linthicum, MD

Reference Sites

1. Shopping Center - Beltsville, MD
2. School playground - Beltsville, MD
3. Route 1 - Beltsville, MD

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suggested by May (1967) for outdoor sampling, the samples were obtained by drawing air through the sampler at the rate of 28.3 L/min. Indoor samples were obtained at the same flow rate but with the sampler in the upright position and cone in place. Sample time was adjusted so that overloading of colonies on plates would not occur and, thus some samples were as short as 15 s (mushroom house) and others as long as 30 min (reference sites). Samplers were always cleaned and autoclaved between samplings, and assembled and disassembled in a laminar-flow hood to prevent extraneous contamination.

EXPERIMENTAL PROCEDURES AND MATERIALS FOR THE LABORATORY

Fungi and Thermophilic Actinomycetes in Air

Colonies that appeared on sampler plates incubated 24 h at 45°C were used to determine counts of *Aspergillus fumigatus* (AF), and those incubated at 50°C were used to determine counts of thermophilic actinomycetes (TA). Colonies were counted at 24 h, and the number of particles bearing viable microbes per unit volume of air sampled was calculated according to the expression: $\text{Cfu/m}^3 = (\text{total number of colonies on six stages}) \times (0.028\ \text{m}^3/\text{Min.}) \times (\text{min. sampled})$. Identifications of AF were based on microscopic and colony morphology as described by Raper and Fennell (1965). Identifications of TA were also based on microscopic and colony morphology using the criteria and descriptions presented in Cross (1968), Goodfellow and Cross (1974), and Lacey (1973). In addition, characteristics of the air sample isolates were compared with those of the reference strains (Table 16).

Isolates identified as *Streptomyces* sp. on the basis of their micromorphological characteristics were studied further to determine their affinity to some of the above listed species. In this regard, tests for melanoid pigment production on peptone iron agar and utilization of D-glucose, L-arabinose, D-xylose, i-inositol, D-mannitol, D-fructose,

TABLE 16. REFERENCE STRAINS OF ACTINOMYCES USED IN CHARACTERIZATION STUDIES

Isolate Name	Culture Collection or Source Number
Micropolyspora faeni	NRRL-8014
Saccharomonospora viridis	T-128 (from V. Kurup)
Streptomyces albus	NRRL-2208
S. eurythermus	NRRL-2539
S. griseoflavus	NRRL-5312
S. griseus	NRRL-2926
S. macrosporeus	ATCC 19783
S. thermodiastaticus	NRRL-5316
S. thermoflavus	NRRL-12503
S. thermonitrificans	ATCC 23385
S. thermophilus	ATCC 19282
S. thermotolerans	NRRL-2345
S. thermoviolaceous	ATCC 19283
S. thermovulgaris	ATCC 19284
Thermoactinomyces candidus	ATCC 27868
T. vulgaris	NRRL-5790

sucrose, rhamnose, and raffinose as sole sources of carbon were done according to procedures described by Shirling and Gottlieb (1966). Ability to grow at 25°C was determined on trypticase soy agar. Tests for gelatin liquefaction, nitrate reduction, and starch hydrolysis were done in accordance with standard methods (Lennette et al., 1974).

Nutritional and Non-Nutritional Factors Affecting the Growth of *Aspergillus fumigatus* (AF)—

Tolerance limits on factors which affect the germination, growth, and sporulation of AF were investigated so that practical modes of imposing environmental stress for the organism in the composting situation might be suggested. Factors which might be controllable in large-scale composting operations include the use of various bulking agents and moisture, temperature, and oxygen/carbon dioxide control.

Substrates—Reports have indicated that sapwood of birch, spruce, and pine support growth of AF (Kaarick, 1974). Because composting with woodchips involves many species of woody plants the suitability of different species as substrate was studied. Freshly chipped wood samples and oak leaves from several tree species obtained in the Beltsville, MD area were point inoculated with 0.5 mL of a water suspension containing approximately 10^4 AF spores/mL. Spores were obtained by flooding a 7 d, 45°C, potato dextrose agar culture of AF isolate No. 267 with sterile distilled water. The woodchips and leaves (20 g) were incubated in sterile, moist chambers over water for 7 d at 45°. Initial and final counts of AF on chips were determined by dilution plating of samples onto oxgall-gentamicin agar with incubation at 45°C.

The possibility of using various bulking materials and filter press cake sludge in the composting of sludge suggested that they be tested for the ability to support growth of AF. Potential bulking agents and filter-press cake were incubated and assayed for AF as described above for woodchips, except that the incubation was 14 d for bulking agents and 2, 4, 8, and 12 d for filter-press cake sludge.

Temperature Effects on Growth—Seven AF isolates were selected for study and they represented the range of colony morphological features found among the several hundred isolates obtained from sewage-sludge compost and compost-site air samples. The isolate numbers and colony characteristics are listed below.

Isolate No.	Colony Characteristics*
276, 399A	Abundant, greyish-green sporulation
267A	Weak, latent, greyish-green sporulation
335A, 338A	Abundant, mouse brown sporulation
660B-1, 660B-3	Abundant, greyish-green sporulation with red diffusible pigment in the medium

* on Potato Dextrose Agar at 45°C.

The optimum and maximum temperatures for growth of the seven isolates were determined by measuring the increase in linear hyphal dimension on nutrient agar (NA) incubated at 25, 28, 35, 40, 45, 50, and 55°C. The linear phase of fungal growth occurs at a constant rate, K_r, known as the radial growth rate. The K_r is a reliable measure of the effect of an environmental parameter, such as temperature, on fungal growth, provided that the parameter does not substantially alter the width of the peripheral growth zone of the fungal colony (Trinici, 1971). Temperature does not alter this zone.

In separate tests, the effects of the nutrient medium and the temperature on growth rate of isolate No. 267 were studied. Media used were potato dextrose agar (PDA), PDA with SAP, water agar, compost (5% w/v) agar with SAP, and sludge (5% w/v) agar with SAP. The compost that was used was a 1 cm fraction from finished compost obtained from the Beltsville Agricultural Research Center Compost Site. The sludge was fresh primary sludge from Blue Plains Wastewater Treatment Plant, Washington, D.C. All agar mixtures were adjusted to pH 7.5 with HCl or NaOH before sterilizing. After autoclaving and when cooled to 50°C the filter-sterilized SAP (streptomycin, aureomycin, and penicillin) solution was added to the agar to prevent bacterial contamination. In preliminary experiments, this was found necessary for the sludge and compost agar. The final concentration of SAP in the agar was 50 ppm streptomycin, 2 ppm aureomycin, and 10 ppm penicillin.

An electroconductivity test was done on the sludge and compost to determine the soluble salts present. Samples of 5 g (dry wt.) were mixed with 20 mL of deionized, distilled water. These were shaken for 1 min in a 30 cm arc, allowed to settle for 30 min, filtered through Whatman No. 1 filter paper. Conductivity of the filtrates was measured in a Beckman Conductivity Bridge. Pellet inoculum for the test dishes was prepared by growing spore suspensions of the test isolates in nutrient broth on a reciprocating water-bath shaker for 48 h at 40°C. The nutrient medium had the following constituents (g/L deionized distilled water):1.42, $Na_2HPO_4 \cdot 7H_2O$; 0.75, KH_2PO_4; 0.10, NaCl; 0.12, $MgSO_4 \cdot 7H_2O$ 0.40, NH_4NO_3; 1.8 glucose; 0.10, yeast extract. Each petri plate (90 mm diam) containing approximately 20 mL of nutrient agar (NA), prepared with the above components plus 2% agar, was inoculated centrally with a single mycelial pellet; triplicate plates of each isolate were incubated at the seven test temperatures. Colony diameters were measured daily along two perpendicular axes and the measurements were averaged for each plate, thus three measurements of colony diameter were obtained for each isolate at each temperature. The constant radial growth rate K_r, was calculated for each isolate at each temperature by linear regression using all measurements of diameter except those between the time of inoculation and initiation of a linear growth phase (determined graphically). The slope derived from the linear regression was designed K_r. The day on which a K_r first occurred for an isolate at a specific temperature was noted also.

Effect of Gaseous Environment and pH on Growth--The tolerance of AF to low oxygen tension was studied because of the possibility of controlling aeration levels in the compost pile. Three test conditions were used.

First, a vacuum dessicator containing 3 petri plates each of corn-meal agar (Difco) and trypticase soy agar (Difco) inoculated in the center with 0.5 mL of a water suspension of AF spores (obtained as described above) containing 10^5 AF spores/mL was prepared by alkalinating pyrogallol and flushing with N_2. Plates were incubated in the dessicator 5 d at 30°C.

In the two other tests, atmospheres were adjusted to 5% O_2/5% CO_2 and 0.5% O_2/23% CO_2 with incubation of the chambers at 45°C for 48 h. Triplicate plates of media inoculated as described above included avicell cellulose agar, corn meal agar, and oxgall agar. Oxygen and CO_2 levels were determined in triplicate in a Beckman gas analyzer.

Sewage sludge is often extremely alkaline (pH 12) as a result of lime (CaO) addition at the end of wastewater treatment processing. During the initial stages of composting the extreme alkalinity is reduced toward neutrality. The influence of these broad changes in pH on the growth of *A. fumigatus* was determined by comparing the constant radial growth rates determined from measurements of mycelial extension on NA media adjusted to different pH levels. The NA medium described above was used for pH studies but was buffered (0.01M) at pH 6 with BIS TRIS propane (1, 3 bis(tris-(hydroxymethyl)methylamino)propane), at pH 7 with TES (N-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid), at pH 8 with TRICINE (N-tris-(hydroxymethyl)methyl glycine), at pH 9 and 10 with CHES (cyclohexylamino-ethane sulfonic acid).

Six plates of each of the seven isolates at all pH levels were inoculated with a mycelial pellet prepared as for the temperature study inocula. Three plates of each isolate/pH combination were incubated under 5% CO_2, the other three were incubated under ambient atmospheric conditions (0.03% CO_2), and in both cases the temperature was 37°C. Colony diameters were measured daily and constant growth rates were calculated as described for temperature data.

Effect of Osmotic Potential on Growth--Sewage-sludge compost frequently has a high concentration of salts because the sludge itself is highly limed and supplemented with $FeCl_3$ during wastewater treatment processing. The salts content will influence the availability of water to biological entities growing in the sewage-sludge compost. The influence of water potential on the growth of *A. fumigatus* was tested by comparing growth rates on NA media prepared as described above but with the addition of increasing amounts of KCl to establish different water potentials in the media.

A suspension of spores from isolate No. 267 was grown for 48 h in shake flask containing the basal medium of Sommers et al. (1970). This basal medium was composed of $Na_2HPO_4 \cdot 7H_2O$, 1.42 g; KH_2PO_4, 0.75 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; NaCl, 0.10 g; NH_4NO_3m 0.43 g, glucose, 1.80 g; yeast extract, 0.10 g; malt extract, 1.00 g and 1L of deionized, distilled water. A mycelial pellet from the shake flask was used to inoculate the center of each plate that contained approximately 18 mL of the osmotic-potential-adjusted agar medium. The different osmotic potentials were obtained by addition of different amounts of KCl to the

Sommers basal medium containing 2% agar. The concentrations of KCl used were determined by calculation from the data of Campbell and Gardner (1971).

The modified van't Hoff relation was used to calculate the model concentrations of KCl and the resulting osmotic potentials in bars from Campbell and Gardner's data (1971).

= osmotic coefficient 1
= universal gas constant (0.0831 bar kg $mole^{-1}K^{-1}$)
= osmotic potential (-bars)
= absolute temperature (°K)
= number of osmotically active particles per molecule of solute.

Finally, osmotic potential was expressed in terms of Pascals (10^5Pa=1 bar). Table 17 shows the values calculated and used to prepare agars with different osmotic potentials.

TABLE 17. KCl CONCENTRATION AND CORRESPONDING POTENTIALS (kPa X 10^2) FOR BASAL MEDIA CONTAINING DIFFERENT AMOUNTS OF KCl.

KCl (Molal)	g KCl/L	Osmotic Potential[1]			
		25°C	30°C	37°C	40°C
0.32	23.86	16	16	16	16
0.63	46.97	29	30	30	31
0.95	70.83	43	44	45	46
1.26	93.93	57	58	60	60
1.58	117.79	71	73	74	75
2.22	165.50	100	102	104	105
2.84	211.72	127	130	133	134
3.45	257.20	154	157	161	163
4.07	303.42	182	185	190	192
4.68	348.89	209	213	218	220

Basal Medium Alone - 1.2 kPa x 10^2

[1] Based on osmotic potential of basal salts solution with added KCl.

RESULTS

Natural Airspora of A. fumigatus (AF)

The airspora of the different sample sites during each season are shown in Table 18. During winter the airspora in outdoor locations was generally lower than that during other seasons. Some of the suspected AF sites proved to have very low airborne levels, e.g. lawn during mowing under trees and in parks and around a hospital. Also, the wooded areas proved to have very low levels of AF as did the refuse areas, the cornfield, and the brush pile. AF levels at the reference sites were also lower in winter than in summer, and were never greater than 12 cfu/m^3. The mushroom house had the highest amount of AF in the air; samples were obtained during the clean-out phase of the mushroom operation. The dust generated during

TABLE 18. 1979-80 NATURAL AIRSPORA OF ASPERGILLUS FUMIGATUS.

Site:	Seasonal counts (colony-forming units/m^3) Fall	Winter	Spring	Summer
Lawn				
during mowing	1	5	2	0
with mulch	75	2	6	686
under trees	3	0	5	4
of hospital	2	0	0	0
of park	8	4	24	2
Wooded Area:				
arboretum	4	1	6	136
nature trail	56	0	10	8
road side	1	5	2	3
Agricultural:				
corn field	1	0	0	4
barn	2,070	105	352	5,550
barnyard	44	0	35	4
poultry house	21	93	2,060	6
mushroom house	88,700	740,000	580,000	67,100
brush pile	1	1	25	5
Refuse:				
municipal dump	6	2	0	5
supermarket dumpster	2	0	0	12
Greenhouse:				
potting room	868	1,350	1,070	9,810
low humidity	NS	11	312	1
high humidity	NS	0	152	2
Library stacks	171	0	0	0
Attic	NS	1	1,160	125
Zoo - birdhouse	5	0	42	2
Boiler Room	30	38	1	1
Reference Sites				
School playground	6	1	12	9
University parking lot	7	1	12	9
Shopping Center	11	1	7	3

NS=not sampled

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clean-out was so great as to nearly block out the outline of the open doors at each end of the mushroom house. During the spring sampling of the attic an old ostrich feather duster was moved around and this dislodged visible fines. The feathers were later found to be contaminated with AF.

Substrate Studies

Several types of wood common to the mid-Atlantic region of the U.S.A., as well as oak leaves, and various bulking agents were tested for their ability to support growth of AF. Results (Table 19) indicate that all types of woodchips as well as oak leaves, paper pellets and cubes, and peanut hulls are excellent substrates for AF growth and sporulation when sufficient moisture and temperature are provided. Shredded newspaper, although cellulosic, supports less sporulation than the other nonpreserved cellulosic substrates tested. The printing inks and low nitrogen content of the newspaper may have inhibited some growth.

In other tests, AF levels remained low, 0-10.5 colony-forming units/g dry weight of filter press cake made from digested sewage sludge and advanced wastewater treatment sludge. During the 44°C incubation of nonsterile samples of the filter press cake the pH remained high, e.e., pH 11.8-12.1 over 12 d. The possible inhibitory effect of high pH on growth and sporulation of AF was considered in further studies reported below.

TABLE 19. GROWTH OF AF ON STERILIZED WOOD CHIPS, LEAVES, AND POTENTIAL COMPOST BULKING AGENTS AT 45°C IN MOIST CHAMBERS.

	Increase (+) or decrease (-) in AF concentration during incubation* (log units)
Woodchips	
Poplar	+5
Red maple	+5
Scotch pine	+4
Sweet gum	+4
Virginia pine	+4
Mixture of chips	+5
Oakleaves	+5
Bulking Agents	
Fly ash	-4
Paper pellets	+3
Paper cubes	+2
Peanut hulls	+2
Pier piling (creosoted wood)	-1
Rubber tires	-1
Salvaged autoseats	0
Shredded newspaper	+1

*Inoculum levels 2.0 x 10^3 spores/g dry weight

Temperature Studies

Constant radial growth rates (mm/d) for one AF isolate on different media is shown in Table 20. Growth rates of seven AF isolates at each of seven incubation temperatures are shown in Table 21. The growth rate with the shortest lag time occurred at 40°C for all seven isolates. At 35 and 40°C isolates 335A and 338A were significantly more rapid growers with shorter lag times than any of the other five isolates. All of the seven isolates grew poorly at 55°C, and at 59°C they all grew very slowly. At 45°C the growth rate was 6 to 8 times greater than that at 50°C. Isolates tended to rank as more rapid growers at certain ranges of temperature. Isolate 335A was more rapid than other isolates at temperatures 35 and 40°C.

TABLE 20. COLONY GROWTH RATE (mm/d) OF ISOLATE NO. 267 ON FIVE NUTRIENT MEDIA AT FIVE TEMPERATURES.

Agar Media	Temperature					Sporulation Index
	25°C	35°C	45°C	50°C	55°C	
Water	7.5*	12.6	8.2	3.7	0	+ 1
PDA	13.6	21.0	8.4	4.2	-	+ 5
PDA & SAP	12.6	21.1	11.3	4.2	0	+ 5
Compost & SAP	7.6	10.4	7.0	2.4	0	+ 1
Sludge & SAP	5.5	8.3	6.1	1.7	0	+ 3

* average of 3 measurements.

TABLE 21. COLONY GROWTH RATE (mm/d) OF 7 _A. FUMIGATUS_ ISOLATES GROWN AT 7 TEMPERATURES.

Isolate No.	Temperature (°C)						
	25	28	35	40	45	50	55
267	9.3 *	13.5	20.5 a**	21.7 b	14.8	4.0	tr***
267A	10.0	11.8	20.3 a	21.8 b	17.8	4.0	tr
335A	7.7	13.3	21.3 b	24.0 d	17.1	2.3	tr
338A	10.3	14.0	21.5 b	24.3 d	18.0	2.7	tr
339A	9.2	11.2	21.1 b	22.7 c	18.3	2.5	tr
660B-1	9.2	12.7	20.0 a	23.5 d	15.5	3.2	tr
660B-3	11.0	11.8	20.0 a	22.0 c	17.8	2.7	0

* average of 3 replicates.
** values followed by the same letters are not significantly different according to Duncan's multiple range test (P = .05).
*** tr = trace amount of growth, not measurable.

Gaseous Environment and pH Studies

Spores of AF germinated on corn meal and trypticase soy agars in 5 d at 30°C under nitrogen gas. However, growth was limited to a sparse hyphal phase and no conidiophores or spores were produced. In the control plates incubated in an ambient atmosphere, abundant sporulation occurred.

In test chambers containing 5% O_2 and 5% CO_2 at 45°C, AF spores germinated in 48 h on oxgall, corn meal and avicell cellulose agars and sporulation was abundant. In test chambers containing 0.5% O_2 and 23% CO_2, AF spores germinated and mycelial extension was equivalent to that observed on the control plates incubated in air at ambient temperature. However, only a very limited number of conidiophores and spores were produced, whereas abundant spores were produced in ambient atmosphere controls.

Results of pH studies (Table 22 and Fig. 38) on AF isolate 267 show that rapid growth and sporulation occur at pH 6, 7, and 8, but decreased growth rate and sporulation are evident at pH 9 and 10.

In other tests (Table 23), growth rates at pH 6, 7, and 8 with 5% CO_2 were comparable for all seven AF isolates. At pH 6 and 7 the growth rates for all isolates incubated at 0.03% CO_2 were comparable to those corresponding to the 5% CO_2 incubations. However, at pH 8 growth rates for the 0.03% CO_2 tests were consistently lower than the corresponding pH 8 tests at 5% CO_2.

TABLE 22. COLONY GROWTH RATE (mm/d) OF AF ISOLATE NO. 267 AT 37°C AT FIVE pH LEVELS

pH	Growth rate (mm/d)*	Sporulation on day no.
6	16.0	2
7	24.0	2
8	21.5	2
9	14.7	3
10	1.9	No Sporulation

*Calculated from the change in colony diameters between days 3 and 4 of the incubation.

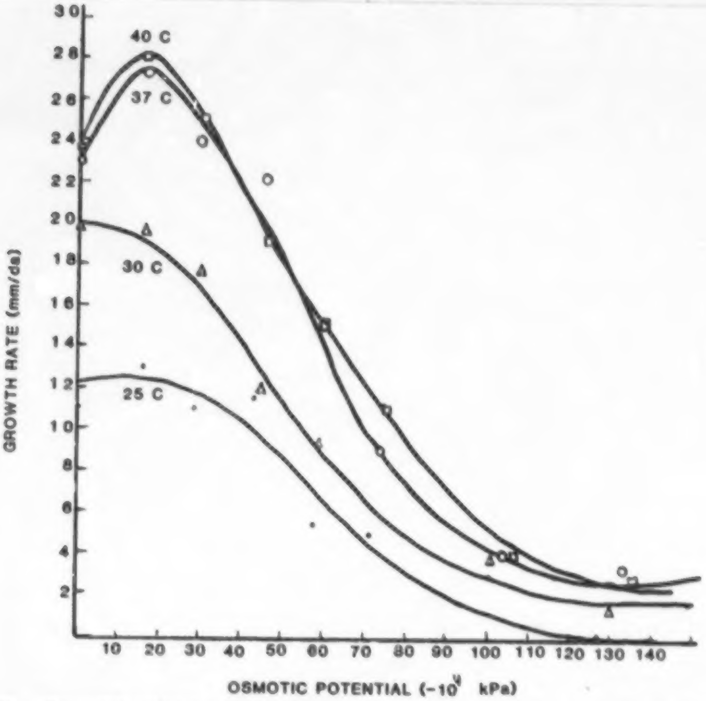

Fig. 38. Growth rate of A. Fumigatus at four temperatures and eight osmotic potentials.

TABLE 23. GROWTH RATE (mm/d) OF SEVEN ISOLATES OF AF AT THREE pH LEVELS AND TWO LEVELS OF CO_2 .*

	5.0% CO_2			0.03% CO_2		
Isolate No.	pH 6	pH 7	pH 8	pH 6	pH 7	pH 8
267	16.5	17.2	17.2	17.5	17.5	14.5
267A	16.4	16.7	16.3	16.8	17.2	14.9
660B-1	17.4	17.8	17.7	18.3	17.6	13.0
660B-3	16.8	16.6	16.8	17.3	17.2	14.6
335A	17.9	18.6	17.6	19.5	18.3	13.7
338A	16.8	17.0	16.8	17.8	17.7	12.5
339A	15.7	16.0	16.7	18.1	17.0	13.7

*Values are the mean of three replicates.

Osmotic Potential

The effects of osmotic potential on growth of AF at different temperatures are shown in Table 24 and Fig. 39 as was shown in the temperature studies maximum growth occurs at 37 - 40°C. Decreased growth rate occurred at all temperatures when the osmotic potential of the medium was less than -40×10^2 kPa. Moisture release curves (Fig. 39) on compost done by the thermocouple pyschrometry method (Campbell and Gardner, 1971) indicate that -40×10^2 kPa would be associated with a water content of less than approximately 30% (W/W). Such a low water content is very unlikely for microbially active compost of microbiological processing. There was no growth at KCl molalities of 3.45, 4.07, or 4.68, except for trace growth at 3.45 m at 37°C on day 7.

Natural Airspora of Thermophilous Actinomycetes

Results of the seasonal studies of the actinomycete flora at different sites are shown in Table 25. Most outdoor and indoor locations have less than 10 thermophilous actinomycete colony-forming units/m^3. Exceptions included the mushroom house, barn, barnyard, and poultry house. The mushroom house was sampled during the clean out phase in which spent compost and casing soil are manually removed from the stationary mushroom beds. During sampling in the barn, barnyard, and poultry house, normal animal activity was in progress, but no other moving of hay or straw occurred by machines or workers. *Thermoactinomyces* spp. and *Saccharomonospora viridis* were isolated from the agricultural sites. Farmer's lung type actinomycetes were not obtained from any of the other sites, except the greenhouse potting room. Thermophilous actinomycetes from other locations belonged to the genus *Streptomyces* and were identified according to criteria established by Lacey (1973) and Goodfellow and Cross (1974).

Identification of Thermophilous Actinomycetes Obtained from Air

Study of type strains of *S. eurythermus*, *S. griseoflavus*, *S. macrosporeus*, *S. thermodiastaticus*, *S. thermoviolaceus* ssp. *violaceus*, and *S. thermovulgaris* indicated that two* of the four categories of characteristics used for identification of *Streptomyces* species (color reactions on nutrient media*, sporophore morphology*, spore surface morphology, and

TABLE 24. COLONY GROWTH RATE (mm/d) OF *A. FUMIGATUS* AT DIFFERENT KCl CONCENTRATIONS AND TEMPERATURES.

KCl Molality	25°C	30°C	37°C	40°C
0.00	11.0	19.5	23.5	23.5
0.32	13.0	19.5	27.5	28.0
0.63	11.0	17.5	24.0	25.0
0.95	11.5	12.0	22.0	19.0
1.26	5.5	9.5	15.0	19.0
1.58	5.0	7.0	9.0	15.0
2.22	3.0	4.0	4.0	11.0
2.84	Trace	1.5	3.5	4.0

TABLE 25. 1979-80 NATURAL AIRSPORA AT THERMOPHILOUS ACTINOMYCETES

Site	Seasonal counts (colony-forming units/m^3)			
	Fall	Winter	Spring	Summer
Lawn:				
during mowing	2	2	7	0
with mulch	0	0	0	1
under trees	0	0	5	2
of hospital	0	5	0	1
of park	0	8	2	12
Wooded Area:				
arboretum	5	0	5	5
nature trail	0	2	4	5
road side	0	1	4	0
Agricultural:				
cornfield	2	2	1	5
barn	NS	18	0	5
barnyard	NS	132	1	51
poultry house	0	29	43	1
mushroom house	204	24,600	35,800	3,470
brush pile	1	4	10	5
Refuse:				
municipal dump	1	4	2	6
supermarket dumpster	1	1	0	1
Greenhouse:				
potting room	13	0	1	0
low humidity	NS	2	12	2
high humidity	NS	0	4	0
Pool - indoor	11	0	3	1
Library-stacks	0	2	4	7
Attic	NS	0	2	4
Zoo-birdhouse	5	1	4	8
Boiler room	4	0	0	1
Reference Sites:				
School playground	3	3	3	3
University parking lot	2	1	2	2
Shopping Center	2	2	3	3

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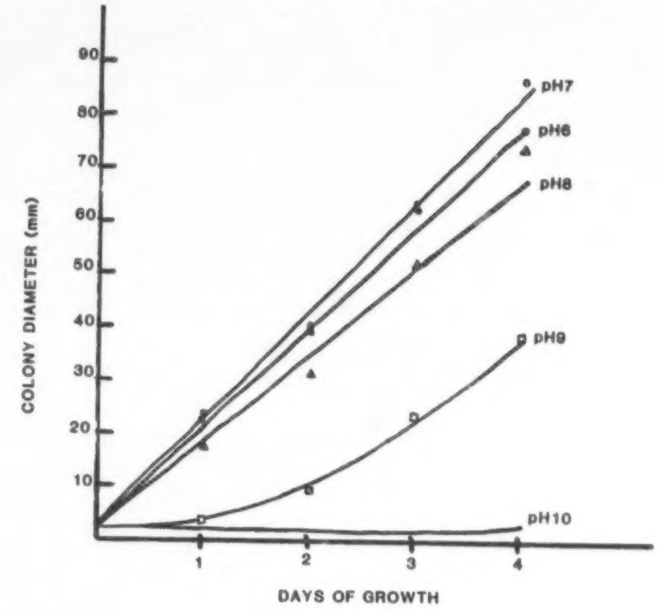

Fig. 39. Growth rates of A. Fumigatus at five pH levels.

carbohydrate utilization pattern) are nondiscriminant for thermophilic isolates. The ambiguity of the sporophore morphology in S. macrosporeus, S. thermodiastaticus, and S. thermonitrificans was noted also from ISP descriptions (Shirling and Gottlieb 1976).

An identification scheme for thermophilous Streptomyces was devised on the basis of test results from the type and 55 compost and 35 natural airspora strains. Production of melanoid pigmentation, spore size, spore surface morphology, and nitrate reduction appear to be useful characteristics for differentiation of this group. Patterns of carbon utilization are considered less valuable than the above characteristics, particularly morphology, because of the variable reactions obtained with type strains. In contrast, spore morphology is relatively stable and a composite result of multi-genic interactions not easily responsive to one gene-one enzyme changes. The following scheme was used to differentiate the Streptomyces species which produced grey aerial mass color on soluble starch-salts agar with growth at 50°C.

1. Melanoid pigmentation produced on peptone iron
 agar or tyrosine agar 2
1. Melanoid pigmentation not produced 3
 2. Nitrate reduced to nitrite, spore surface spiny
 and smooth, sporophores rectus-flexibilis (RF)....... S. thermophilus

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2. Nitrate not reduced to nitrite, spore surface smooth, sporophores RF, rectinaculum-apertum (RA), or spira(S) .... *S. eurythermus*
3. Spores large, diameter _ 1.5 um, spore surface spiny, sporophores RF and S .... *S. macrosporeus*
3. Spores smaller than 1.5 um diam., spore surface warty, spiny, or smooth, sporophores RF, RA, S or irregular 4
4. Spores separated by short hyphal sections, spores spiny, sporophore branches irregularly rectangular .... *S. fragmentosporus*
4. Spores not separated by hyphal sections 5
5. Spore surface warty or spiny 6
5. Spore surface smooth 8
6. Nitrate reduced to nitrite; arabinose and mannitol utilized; sucrose, rhamnose, and raffinose utilization variable 7
6. Nitrate not reduced; arabinose utilization variable; mannitol, rhamnose, sucrose and raffinose not utilized .... *S. thermoviolaceus*
7. Xylose, inositol, fructose and raffinose utilized; sucrose not utilized; sporophores RA and S .... *S. thermodiastaticus*
7. Xylose, inositol, fructose and rhamnose utilized; sucrose and raffinose not utilized; sporophores RA and S .... *S. griseoflavus*
8. Sporophores RF, RA, and S; rhamnose and raffinose utilized; nitrate reduced to nitrite..... *S. thermovulgaris*
8. Sporophores RF and S; rhamnose and raffinose not utilized; nitrate reduced to nitrite .... *S. thermonitrificans*

There are some compost isolates which are not accommodated by this key. They would appear as *S. thermoviolaceus* in the above scheme, except for their variable patterns of carbon utilization. This group of isolates will require further study in order to determine its proper disposition. A numerical taxonomy approach would be useful here.

DISCUSSION

Experimental

Natural Airspora--

In general, the AF content in air at the tested reference sites and at most other suspected sites is less than about $20/m^3$. Such levels are comparable to those reported by other investigators in Great Britain and the USA (Table 14). Some agricultural sites, such as barns, barnyards, poultry and mushroom houses are natural sources of larger amounts of airborne AF.

Thermophilous actinomycetes (i.e., isolates capable of growth at 50°C) were not abundant in air at the reference sites or at the nonagricultural sites. Those that were obtained in air from nonagricultural sites are *Streptomyces*, especially *thermoviolaceus* and *S. thermovulgaris*. Most of the non-streptomycete actinomycetes obtained from the agricultural sites were Thermoactinomyces, an agent of farmer's lung.

Nutritional and Nonnutritional growth factors--

Factors important to the growth and sporulation of AF include type of substrate, temperature, CO_2 level, pH, and water potential. One strategy for potentially decreasing the growth of AF during composting is to manage the composting process so that conditions unfavorable to AF are achieved. The practicality of such a management process relies on the combined requirements of the composting process and the growth limits of the fungus.

Use of substrates other than woodchips and cellulose-containing materials as bulking agents is very likely to substantially reduce the growth of AF during composting. Reduction in AF would be aided by dry storage of bulking agents. As presently practiced, storage of woodchips in piles prior to mixing with sludge leads to proliferation of AF in the storage piles. Thus, the compost is inoculated with AF. Reduction in inoculum levels by use of alternative bulking agents should assist in reducing AF at compost sites. This conclusion is further supported by the fact that sludge and compost are relatively poor growth substrates for AF (Table 20). Also, the initially high pH of sludge would inhibit AF growth (Table 22).

In contrast with the substrate and pH, control of temperature, CO_2, and water content are less likely to lead to reduced levels of AF. The fungus tolerates a broad range temperature (Tables 20 and 21), CO_2 (Table 23), and reduced water availability (Table 24). Limiting AF growth by controlling the composting process through any of these factors is not practically desirable or achievable.

SUMMARY

Results of these studies have shown that AF and thermophilous actinomycetes (TA) are present in air at very low levels in most non-agricultural sites even if organic matter and occassionally higher than ambient temperatures are present also. Thus, the natural sources for AF and TA are very limited in terms of abundant production of spores. Few spores are airborne from the natural sources even during mechanical disturbances such as mowing. High levels of AF and TA in air are associated with heavily colonized substrates. Compost and moldy agricultural substrates are the biggest reservoirs and sources of AF and TA.

Practical alterations of the sewage-sludge composting process to reduce AF growth and dispersal from compost sites presently should focus on use of noncellulosic bulking agents. Such agents would substantially reduce the inoculum levels entering the process. The tolerance of AF to broad ranges of temperature, CO_2, and water content limit the benefits of modifying the composting process by these parameters to inhibit AF.

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APPENDIX A

SPECIFICATIONS FOR DIFFERENTIAL TEMPERATURE CONTROLLER

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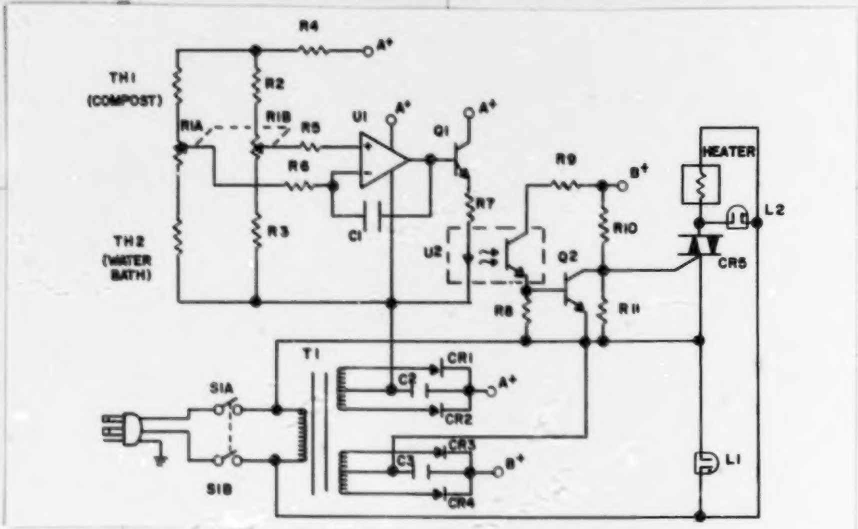
R4
A+
TH1 (COMPOST)
R2
R1A
R1B
R5
U1
A+
Q1
A+
R6
R9
B+
HEATER
L2
R7
C1
R3
TH2 (WATER BATH)
U2
Q2
R10
CR5
R8
R11
T1
CR1
C2
A+
CR2
S1A
S1B
CR3
C3
B+
CR4
L1

Fig. A-1. Circuit diagram of differential temperature controller. See Table A-1 for key to components.

TABLE A-1. SPECIFICATIONS OF COMPONENTS IN DIFFERENTIAL TEMPERATURE CONTROL SYSTEM

Designations	Component	Specification
TH 1, TH 2	thermistor	5000 Ω at 25C Omega* 44007
R1	dual potentiometer	50 Ω
R2, R3	resistor	178 Ω, .25W
R4, R8	resistor	1k Ω, .25W
R5, R6	resistor	100k Ω, .25W
R7	resistor	470k Ω, .25W
R9	resistor	4.7k Ω, .25W
R10	resistor	150 Ω, 2W
R11	resistor	150 Ω, .5W
U1	operational amplifier	RCA* 3160
Q1, Q2	transistor	2N2222
U2	optoisolator	4N28
T1	power transformer	Stancor* P8680
L1, L2	pilot light	neon
CR5	triac	2N 6145
C1	capacitor	0.01 μF
C2, C3	capacitor	500 μF
CR1-4	diodes	1N4000

*The use of trade names is for the convenience of the reader and does not imply endorsement by the USDA or EPA over similar, available products.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

PAGE NUMBER
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